Probes, Methods of Making Probes and Applications of Probes

TECHNICAL FIELD

[01] The present invention relates to methods and apparatuses for analyzing molecules, particularly polymers, and molecular complexes with extended conformations. In particular, the methods and apparatuses are used to identify sequence information in molecules or molecular ensembles, which is subsequently used to determine structural information about the molecules. Further, the present invention relates to forming probes and films for making such probes.

BACKGROUND ART

[02] Twenty-first century science and technology endeavors, research and development innovations that solve problems for man-kind will increasingly be dominated by the ability to make structures and objects that have sizes with length scales approaching those of atoms and molecules having dimensions of a nano-meter or less. Nano-scale matter and objects exhibit unique behaviors, some of which have yet to be unraveled in addition to the known remarkable optical, thermal, electrical and mechanical properties. These open new vistas for many beneficial applications making them suitable for many applications. For example, sequencing, imaging, nano-lithography, manipulation, nano-scale self assembly, nanometer scale chemistry, and infinite other applications with benefit from nano-scale technology development.

[03] It is envisioned and believed that being involved in the nano-size frontier of science , technology and innovation is a sure path to regional and national economic well being, and competitiveness. This is evidenced by the extraordinary

investment activities by big and small countries, large and small private sector enterprises and nearly unparalleled entrepreneurial activities.

[04] To advance in the nano-scale frontier science and technology requires access to and mastering the following:

[05] Tools to produce nano-objects

[06] Tools to measure sizes with sub-Angstrom precision

[07] Substrates that have atomic smoothness with minimum contamination

[08] Tools to see (image) nano-objects and manipulate them, grabbing, moving, gluing, etc.

[09] Nano funnels/nozzles/probes for dispensing substances and stimuli

[10] Tools to accurately measure all physical properties , thermal, electrical , optical,

[11] Key parameters become smaller by 10 to 20 orders of magnitude of quantities accustomed to in the macro-world.

[12] In the last 5 years the collective achievements of the best and brightest people around the world related to the above tools have grown at astonishing rates, delivering numerous discoveries, innovations, methods, products and tools.

[13] One area that could tremendously benefit from nanotechnology is the development of high-throughput DNA sequencers in the 1990's have helped launched the genomic revolution of the 21st century. Almost on a monthly basis, one research group or another is announcing the complete sequencing of a biologically important organism. This has allowed researchers to cross reference species, finding shared and/or similar

genes, and allowing the knowledge of molecular biologists in all the various fields to come together in a meaningful way.

[14] However, current techniques in DNA sequencing are far too tedious, tying up the valuable time of researchers. Even the fastest, most advanced DNA sequencers can at most process a few hundred thousand base pairs a day. The Human Genome Project took over 10 years to complete, indicating that current DNA sequencing technology still has a long way to go before it can be used as a diagnostic tool. Considering that there are about 3 billion DNA base pairs in the mammalian genome, and current sequencing technology is capable of sequencing about 2 million DNA base pairs per day, it would still take over 4 years to sequence the human genome.

[15] Known nucleic acid sequencing methods are generally based on chemical reactions that yield multiple length DNA strands cleaved at specific bases. Alternatively, other known nucleic acid sequencing methods are based on enzymatic reactions that yield multiple length DNA strands terminated at specific bases. In either of these methods, the resulting DNA strands of differing length are then separated from each other and identified in strand length order. The chemical or enzymatic reactions, and the methods for separating and identifying the different length strands, usually involve repetitive procedures. Thus, there remains significant limitations on the speed of DNA sequencing using conventional technology.

[16] Despite these limitations, an incredible collaborative heroic effort was undertaken for the Human Genome Project. It took many years and billions of dollars to obtain the sequence to the human genome. It would be highly desirable to provide a method and system that reduces the time and effort required would represent a

highly significant advance in biotechnology. Indeed, frontier advances are required to increase the efficiency and speed of DNA sequencing if we are to expand the genome databases that presently exist to include a genome library including flora and fauna. Certain flowering plants have 100 times more base pairs than the human genome, so existing sequencing technology must be leaped for a new frontier of sequencing systems.

[17] Pores

[18] One particular type of sequencing method relies on passing strands of DNA through pores. For example, U.S. Patent Nos. 5,795,782, 6,015,714, 6,267,872, 6,362,002 6,428,959 6,465,193 6,617,113, 6,627,067, 6,673,615, 6,746,594 6,870,361 describe various sequencing techniques and apparatus based on pores and flow of DNA fragments through pores. In general the prior art pores have thickness that cannot directly resolve with high spatial resolution without some other indirect deconvolution of the date resulting from changes in ionic conductivities. It further cannot be used for large DNA fragments. Further, it is very time consuming. In general, for an ultra fast DNA sequencing system, there are many limitations with pore based systems.

[19] Therefore, it would be desirable to provide an improved system and method of analyzing extended objects such as linear polymers (including proteins, DNA and other biopolymers).

OBJECTS AND BRIEF SUMMARY OF THE INVENTION

[20] The present invention teaches new methods, devices and tools that advances the nanotechnology art listed above. By departing from methods of prior art

and adding new techniques to improve prior art, the teachings of the present invention result in:

[21] The ability to make free standing nano-thickness atomically smooth films, including single or multiple layers from graphene , mica, and from other layered materials.

[22] These atomically smooth layers can be used as substrates for nano-precison tools

[23] Novel methods to handle the layers, the low cost the production of open and closed nano-probes, funnels, tweezers become possible.

[24] The thickness of the layers are used advantageously to defines the nano-scale dimensions of objects.

[25] The nano-probes in combination with other elements, are used to make tools nano-scopes, to recognize and analyze objects

[26] The novel tools exceed the capability of AFM and STM in the their ability to sequence DNA, RNA more rapidly

[27] Novel nano-lithography tools are produced using the thicknesses of the thin-film layers to define the smallest dimension.

[28] Accordingly, in one aspect of the invention an object is to produce single mono-atomic layers of graphene or mica and other layered materials conveniently and inexpensively. Another object of this aspect of the invention to separate or exfoliate single mono-atomic layers from layered materials such as graphite, mica, dichalgoenides, and attaching them to substrate through a releasable bond.

[29] In another aspect of the invention, an object is to produce atomically smooth layers of metals, insulators, semiconductors, organic and bio-molecular layers.

[30] In another aspect of the invention, an object is to produce and manipulate fibers, organic and bio polymer, nano-tubes and other structures.

[31] In another aspect of the invention, an object is to make alternating heterogeneous layers.

[32] In another aspect of the invention, various probes are formed having tip active area dimensions that are a measured based on a film thickness during manufacturing.

[33] In another aspect of the invention, various probes sets and arrays are formed using the above mentioned probes.

[34] In another aspect of the invention, method of analyzing extended objects are provided using the herein described probes, probes sets and probe arrays.

[35] For example, using the herein nano-nozzles, a DNA sequencing method is presented that may sequence the entire Human Genome in a matter of minutes. Realizing and optimizing this technology opens new vistas for human endeavors, and enables practical applications that are nearly limitless. Culturing bacteria would be a thing of the past. Whenever faced with an unknown organism, not only could its exact species be determined immediately, but also its entire genotype, including new mutations or signs of genetic engineering. This process is based on utilization of the nanoscale probes, e.g., in the form of electrodes, nozzles, funnels, or other suitable probes. These nanoscale probes are coupled with detection of ultra small and ultra fast signals. This

sets the course for the development of the ultimate sensor, not only for DNA, and RNA, but also to sequence denatured proteins (amino acid sequence of polypeptides).

[36] As discussed above, current DNA sequencing technology is most often based on electrophoresis and polymer chain reaction (PCR). PCR is used to create varying lengths of the DNA in question, which is then subjected to electrophoresis to resolve the size differences between the DNA fragments. However, this technique faces several bottlenecks. First, although PCR is useful in amplifying the amount of DNA material, it is time consuming, requires numerous reagents, including the use of an appropriate primer. Second, electrophoresis speed is dependent on the applied voltage. But the applied voltage cannot be further increased unless heat dissipation is similarly increased. Also, electrophoresis gel is only capable of resolving a small dynamic range (<500bp). This requires splitting an organism's genome apart for sequencing and then re-assembling the pieces.

[37] Instead of relying on electrophoresis to resolve the DNA sequence, the proposed sequencing technology is based on nano-electronics.

[38] The herein system and method relies on probes having resolution capabilities less than the dimensions of the objects to be analyzed. Further, systems and methods are provided herein that allow for accurate measurement of the portions of the specimens to be analyzed, such as individual monomers in a polymer chain.

BRIEF DESCRIPTION OF THE FIGURES

[39] The foregoing summary as well as the following detailed description of preferred embodiments of the invention will be better understood when read in conjunction with the appended drawings. For the purpose of illustrating the invention,

there is shown in the drawings embodiments which are presently preferred. It should be understood, however, that the invention is not limited to the precise arrangements and instrumentalities shown. In the drawings, where:

DETAILED DESCRIPTION OF THE FIGURES

[40] Described herein is a novel system and method for analyzing extended object specimens. The system includes analytical probes configured and dimensioned such that the edge of the probe has a thickness direction that is spatially smaller than the desired resolution. Further, in certain embodiments, the analytical probe has a width dimension that is much larger than the thickness of the extended object. In other embodiments, the analytical probe has a path in the width direction that is much larger than the thickness of the extended object.

[41] The "extended object" to be analyzed using the probes described herein may be a complex macromolecule, including complex monomers, polymers, oligomers, dentimers, or other molecules. Examples of such complex macromolecules include, but are not limited to, proteins, polypeptides, peptide- nucleic acids (PNA), having a polypeptide-like backbone, based on the monomer 2-aminoethyleneglycin carrying any of the four nucleobases: A, T, G, or C. In certain embodiments, the polymers are homogeneous in backbone composition and are, e.g., nucleic acids or polypeptides. A nucleic acid as used herein is a biopolymer comprised of nucleotides, such as deoxyribose nucleic acid (DNA) or ribose nucleic acid (RNA). In certain embodiments, the extended object is a single stranded (denatured) DNA molecule with a rigid structure. Other organic or inorganic molecular structures may also be extended objects for the purpose of the present invention whereby these extended objects may be

analyzed, manipulated, physically altered or chemically altered. Further, double stranded structures may be analyzed according to certain embodiments herein, such as double stranded helical DNA strands.

[42] It will be appreciated by one skilled in the art that the system described herein for monomer level resolution may be used for other molecular level detection, e.g., for single small molecules, single monomers, oligomers, or other nano-scale structures.

[43] Further, as used herein, the term "probe" refers generally to any device used to interact with individual portions of the extended object including, for example, individual nucleotides of a RNA or DNA strand, atomic groups an extended object, atomic and molecular bonds and bond interactions, groups of atoms or molecules within the extended objects, and other interactive forces such as covalent bonds, hydrogen bonds, ionic bonds, and other know interactions. Probes may be formed of various configurations and materials to be described further herein.

[44] Further, as used herein, the term "detectable interaction" refers generally to an interaction between the probe and a portion of the extended object. The portion of the extended object with which a detectable interaction occurs may include individual atoms, molecules, or groups of atoms or molecules, and their bonds. The detectable interaction may be in the form of electric field, magnetic field, optical variations, vibration forces, gravitational forces, or other measurable events.

[45] The probes used herein may be formed of various materials and configurations. For example, probes may be in the form of wells, nozzles or funnels (herein after "hollow probes") having a tip for dispensing or holding materials (including

solids, liquids, gases and transition phases) to facilitate analysis of the specimen. Alternatively, the wells or nozzles may be provided in a system and configuration for suction or application of fluid pressure. The nozzles configured for dispensing materials may include conductive inner walls, or a conductive element disposed within a material holding region, in order to facilitate measurement and other voltage applications across the probe. In other examples, the dispensing materials are within a conductive medium to facilitate measurement and other voltage applications across the probe.

[46] Referring now to Figure 2, a continuous edge probe 202 is depicted, for example, in the form of a continuous knife edge. Probe 202 is particularly well suited for analyzing extended object specimens such as biopolymers. Probe 202 is characterized by a tip 204 thickness t, a tip 204 width w, and a height (not identified in the Figure). Importantly, the tip thickness t is dimensioned to obtain the desired resolution of the system. For example, when information regarding individual monomers of a DNA strand is desired, the thickness t should be less than the nucleotide spacing on the strand (about 0.5 nm). Still further, probe 202 has a width dimension w that is preferably much greater than the tip dimension and also much greater than the width of the specimen. In certain embodiments, this width dimension that is much greater than the tip dimension minimizes or eliminates landing error associated with typical probe analysis systems as the probe passes over the specimen. The ratio of *w* to *t* may be, for example, on the order of about 5:1. 10:1, 10s to 1, 100 to 1, 100s to 1, 1000 to 1, 10,000 to 1, or greater depending on the desired application.

[47] These continuous edge probes may be hollow, solid or partly solid and partly hollow.

[48] As shown, in certain preferred embodiments, the probe has a shape that provides a larger end 206 opposite the tip 204. This can, for example, reduce electrical resistance of the probe when end 206 serves as a contact region. Further, the larger end 206 serves to facilitate introduction and dispensation of materials from the probe when the probe is in the form of a nozzle filled with suitable material, as described further herein.

[49] Referring now to Figures 2B and 2C, discontinuous probes 222, 242 are provided. Probes 222, 242 have an elongated width structure with desirably sized tip with, e.g., cutouts or discontinues edge portions. The generalized probes, 222, 242 made according to the present invention can be made in a configurations that several probe sections, 230, 250, which can be accessed independently or together as shown in Figs. 2B and 2C. In certain embodiments, the probe sections 230, 250 serve identical functionality, for example, for redundancy, or to examine plural specimens in parallel. In further embodiments the probe sections 230, 250 serve different functionalities. For example, some applications may require that sub-probe 230a be used for analyzing or sequencing the specimen, adjacent section, 230b used for dispensing substances or stimuli, and section 230c used for imaging or reading alignment marks. In another example, probe section 250a is in the form of an edge with an elongated width as shown, while probe section 250b may be point like probe, as represented in Figure 2C with dotted lines. The probe sections may be functionalized differently to recognize parts of a specimen under test with high degrees of specificity. These discontinuous edge probes may be hollow, solid or partly solid and partly hollow.

[50] Referring now to Figure 3, a probe 302 is depicted. Probe 302 is particularly well suited for analyzing extended object specimens such as biopolymers. Probe 302 is characterized by a tip thickness t, a tip width w, and a height (not identified in the Figure). Further, probe 302 is positioned within a suitable sub-system 308 to impart motion to the probe generally in the direction of the width w along a path pw. Similar to probe 202, the tip thickness t is dimensioned to obtain the desired resolution of the system. The width dimension w of probe 302 is not critical. However, the path width pw is preferably much greater than the width of the specimen. This ensure that as the probe passes over the specimen, landing error associated with typical probe analysis systems is eliminated.

[51] The probes described herein may take on various shapes and functionalities. In certain embodiments, the probes herein have a continuous edge that is closed. In certain embodiments, the probes herein have a discontinuous edge that is closed. In certain embodiments, the probes herein have a continuous edge that is open. In certain embodiments, the probes herein have a discontinuous edge that is open. In certain embodiments, the probes herein have a continuous edge that has some portions along the width *w* of the probe that are closed and some portions along the width *w* of the probe that are open. In certain embodiments, the probes herein have a discontinuous edge that has some portions along the width *w* of the probe that are closed and some portions along the width *w* of the probe that are open.

[52] Note that the probes herein may have a constant cross section along the width *w* of the probe, or in certain embodiments, it may be desirable to provide a

cross section along the width *w* of the probe that is different therealong, for example, with a broader or narrower central portion.

[53] Further, the probes herein may have a constant tip opening or tip active area dimension along the width *w* of the probe. Alternatively, in certain embodiments, it may be desirable to provide a tip opening or tip active area dimension along the width *w* of the probe that is different therealong, for example, with a smaller and larger sections of tip opening or tip active area dimension for different applications.

[54] Additionally, the probes may be formed of a generally inactive body portion, and an active area that forms the tip opening, such as a conductor in the case of closed tip probes, or a tip opening. Alternatively, the body portion may incorporate some other functionality, such as thermal and electrical shielding, precise metrology spacing, or other elements such as micro- or nano-fluidic or micro- or nano-electromechanical devices. Further embodiments will be described herein.

[55] The probes described herein may be formed many different shapes that will provide the desired tip characteristics and dimensions. Figures 3A-3L show various shapes of certain embodiments of probes herein, generally having a closed tip configuration. However, it should be understood that these shapes may also be suitable of any tip configuration and may be incorporated in any continuous edge or discontinuous edge probe described herein.

[56] Figure 3A shows a prismatic shaped probe having a cross section in the form of an elongated tip integral with a triangular region and an elongated rectangular portion at an end opposite the probe tip.

[57] Figure 3B shows a prismatic shaped probe having a cross section in the form of a right triangle, e.g., with the tip flattened.

[58] Figure 3C shows a prismatic shaped probe having a cross section in the form of a trapezoid.

[59] Figure 3D shows a prismatic shaped probe having a cross section in the form of a rectangle.

[60] Figure 3E shows a prismatic shaped probe having a cross section in the form of a triangle, with the tip at the adjoining end of the long sides of the triangle forming the tip for probing or other applications as described herein.

[61] Figure 3F shows a prismatic shaped probe having a cross section in the form of a rectangle with a triangle at the probe tip end, with the tip at the adjoining end of the long sides of the triangle forming the tip for probing or other applications as described herein.

[62] Figure 3G shows a prismatic shaped probe having a cross section in the form of an irregular polygon, e.g., symmetrical about the height axis, with a flat end and with a tip at the adjoining end of sides of the polygon with an acute angle as shown.

[63] Figure 3H shows a probe having a cross section generally in the form of an inverted tear drop, with a tip *t* at the point of the tear drop shape.

[64] Figure 3I shows a probe having a cross section generally in an elongated irregular form, with a tip *t* at an elongated end thereof.

[65] Figure 3J shows a probe having a cross section generally in the form of an ellipse, with a tip *t* at a tangential point elliptical shape at an elongated end thereof.

[66] Figure 3K shows a probe having a cross section generally in the form of a nozzle, such as a "flattened" end of an elliptical or circular cross sectioned tube, with a tip t at the "flattened" end thereof.

[67] Figure 3L shows a probe having a cross section generally in the form of a V-shape, with a tip t at the point of the V-shape.

[68]

[69] Referring now to Figures 4A-4E, probes are shown in various configurations having tip openings t_o, suitable for dispensing and/or holding materials according to the various embodiments herein.

[70] Figure 4A shows a probe having a cross section in the form of an elongated hollow tip integral open to a triangular well region and an elongated rectangular well portion at an end opposite the probe tip with an opening t_o, having a channel therein for holding and facilitating dispensing of materials.

[71] Figure 4B shows a probe having an asymmetrical cross section in the form a rectangle and a truncated triangle forming a probe tip with an opening t_o, having a channel therein for holding and facilitating dispensing of materials.

[72] Figure 4C shows a probe having a symmetrical cross section in the form a truncated triangles forming a probe tip with an opening t_o, having a channel therein for holding and facilitating dispensing of materials.

[73] Figure 4D shows a probe having a symmetrical cross section in the form a angled members forming a probe tip with an opening t_o, having a funneling channel therein for holding and facilitating dispensing of materials.

[74] Figure 4E shows a probe having a symmetrical cross section forming a probe tip with an opening t_o, having a shaped well and a channel therein for holding and facilitating dispensing of materials.

[75] Referring now to generally to Figures 5A-6B, probes having tips, for example, conductive tips, with tip active area dimensions of t, are shown, whereby tips 510, 610, extend beyond the bodies 520, 620, of the structures. As shown, in Figures 5A-5B, a symmetrical probe is provided, and in Figures 6A-6B, a symmetrical probe is provided. Generally, the dimensions a Figures 5A and the dimensions a and b in Figure 6A are greater than the tip dimension t, preferably multiples of the tip dimension t. These embodiments advantageously provide for tips that extend sufficiently far away, for example, to minimize interaction between the probe body, for example, with the specimen or a substrate depending on the application of the probe. This avoids negative effects of substrate material such as accumulation of electrostatic charge and other interfering effects. Referring to Figure 7, an example of an array of probes according to the embodiment of Figure 6A-6B is shown.

[76] Referring now to Figures 8A-8C, views of an open tip probe according to certain embodiments of the present invention is shown, showing an irregular inner channel surface. Figure 8B shows an array of such probes. Figure 8C shows a probe generally as in Figure 8A, wherein only a portion of the inside surface has electrodes 842 therethrough, which may be advantageously in certain applications.

[77] Referring now to Figures 9A-9B, views of an open tip probe according to certain embodiments of the present invention is shown, showing an irregular inner channel surface with differing sub-sections therein. For example, referring to

Figure 9A, a probe is shown having sub-sections that are divided generally along the height dimension of the channel, including sub-sections 912, 914, 916, 918 and 920. For example, sub-sections 912, 914, and 920 may be formed of insulating materials, sub-section 916 formed of conductive materials, and sub-section 918 formed of semiconductor materials. In a further example, and referring to Figure 9B, a probe is shown having sub-sections that are divided generally along the height dimension of the channel, including sub-sections 932, 934, 936, 938 and 930. For example, sub-sections 932 and 936 may be formed of conductive materials, sub-sections 938 and 940 formed of insulating materials, and sub-section 934 formed as an open channel perpendicular to the channel of the probe tip, for example, for providing micro-fluidic operations or other suitable functionality.

[78] In general, variable opening probes may be provided. In certain preferred embodiments, the opening tip dimension is controllable with sub-angstrom precision.

[79] Referring now to Figure 10A a sectional view of a variable tip probe 1010 according to certain embodiments of the present invention is shown, showing an irregular inner channel surface having a fixed section 1014 and a complementary movable section 1016. The movable section 1016 preferably are actuated with angstrom or sub-angstrom precision to define the probe opening 1012. Figures 10B1 and 10B2 shows views looking into the probe opening according to one embodiment, and Figures 10B1 and 10B2 shows views looking into the probe opening according to another embodiment.

[80] Referring now to Figure 11, another embodiment of a variable gap probe 1110 is shown. An actuator 1124 imparts motion to section 1116 of the probe, thereby changing the opening dimension of the tip opening 1112.

[81] Figures 12A and 12B show an enlarged isometric view and side view, respectively, of a probe set 1230 including probes 1242, 1244, 1246, and 1248, and a specimen extended object 1250 upon a platform 1228. In certain preferred embodiments, polymer strand 1250 is a biopolymer such as a nucleic acid (e.g., DNA). Figure 12C shows an enlarged sectional view through any one of probes 1242, 1244, 1246, or 1248. Figure 12D shows a top view of the base platform 1228, showing an exemplary channel 1252. As shown in Figures 12C and 12D, in certain embodiments, a measuring voltage is applied across each probe 1242, 1244, 1246, 1248, and platform 1228, denoted by reference numerals 1254a and 1254b, respectively. As the polymer strand 1250 passes under an activated probe (e.g., a probe with a measuring voltage applied thereto), detectable interactions occur as described in further detail herein.

[82] Figures 13A-13D show a probe set 1330 formed according to embodiments of the present invention. The probe set includes, e.g., a 1 x 4 array (although it is understood that this may be scaled to any size n x m nozzles) of probes 1342, 1344, 1346, 1348.

[83] In certain embodiments, these probes 1342, 1344, 1346, 1348 are in the form of nozzles, e.g., having tips 1354 associated with wells 1356, as shown in Figure 13B and 13C. Generally, the wells having widths in the y direction greater than the widths of the nozzle tips. Figure 13D shows a sectional view of the nozzle array.

[84] The probe set 1330 may be embedded in a body 1358. The material for the probes or nozzles, and the body, may be the same or different materials, and may include materials including, but not limited to, plastic (e.g., polycarbonate), metal, semiconductor, insulator, monocrystalline, amorphous, noncrystalline, biological (e.g., nucleic acids or polypeptides based materials or films) or a combination comprising at least one of the foregoing types of materials. For example, specific types of materials include silicon (e.g., monocrystalline, polycrystalline, noncrystalline, polysilicon, and derivatives such as Si3N4, SiC, SiO2), GaAs, InP, CdSe, CdTe, SiGe, GaAsP, GaN, SiC, GaAlAs, InAs, AlGaSb, InGaAs, ZnS, AlN, TiN, other group IIIA-VA materials, group IIB materials, group VIA materials, sapphire, quartz (crystal or glass), diamond, silica and/or silicate based material, or any combination comprising at least one of the foregoing materials. Of course, processing of other types of materials may benefit from the process described herein to provide probes and bodies of desired composition.

[85] Referring now to Figures 14A and 14B, all probes and probe sets described herein may be configured with respect to the specimen at various angles. For example, referring to Figure 14A, a probe set 1430 may be oriented generally perpendicular (in the length direction) to a specimen 1450. Further, referring to Figure 14B, a probe set 1430 may be oriented (in the length direction) generally at an angle θ with respect to a specimen 1450.

[86] Referring to Figure 14C, a system 1460 is presented whereby the orientation of plural probe sets 1430 relative a specimen 1450 varies. Because the objects of the specimen 1450 (e.g., bases within a DNA strand) may have different orientations, it may be desirable to sequence with a plurality of probe sets 1430. The

plurality of probe sets 1430 may have different angles θ_1, θ_2, θ_3, θ_4, θ_5,... θ_n (e.g., 20° to 160° in suitable increments, arranged sequentially, randomly or in another desirable arrangement. During measurement as described further herein, a controller may determine which orientation of the probe set yields the best signal for a particular base at its inherent orientation. This allows one to measure the data from the probe sets of the array, and determine the optimum signal for certain bases or groups of bases.

[87] In another embodiment, and referring to Figures 14D-14F, the angles of orientation in the height direction may also be varied. For example, referring to Figure 14D, probe set 1430 may be oriented in the height direction generally perpendicular (90°) with respect to the specimen 1450. Further, as shown in Figure 14E, probe set 1430 may be oriented in the height direction generally at an angle ω with respect to the specimen 1450. Referring to Figure 14F, a system 1470 is presented whereby the orientation in a height direction of plural probe sets 1430 relative a specimen 1450 varies. Because the objects of the specimen 1450 (e.g., bases within a DNA strand) may have different orientations, it may be desirable to sequence with a plurality of probe sets 1430. The plurality of probe sets 1430 may have different angles ω_1, ω_2, ω_3... ω_n (e. g., 20° to 160° in suitable increments, arranged sequentially, randomly or in another desirable arrangement.

[88] In another embodiment, and referring now to Figures 15A-15B, the probes according to the present invention may be configured about more than one portion of the specimen to be analyzed, for example, in the form of an extended opening channel which interrogates from more than one side of the specimen.

[89] Presently, it is known to coax DNA fragments through a pore for the purpose of measuring a change in ionic conductivity. Challenges are posed in the consistency of motion through the holes, the resolution, and other interference. The pore is often part of a system of ionic fluids, whereby ionic conductivity change is measured across regions of ionic fluids separated by a membrane and/or layer having one or more pores. For example, as described in the background of the invention, patents 6,870,361, 5,795,782, 6,267,872, 6,362,002, 6,627,067 describe such pores.

[90] However, according to the extended opening channel system 1500 of the present invention, a specimen 1550 is passed through an extended opening channel 1501. Each extended channel opening includes several probes formed according to any one or more of the various embodiments herein. The probes may be configured on one side of the opening, or multiple sides of the opening. In certain embodiments, using an extended opening channel which interrogates from more than one side of the specimen, accuracy may be enhanced, and signal is increased.

[91] As discussed below with respect to Figures 16A-16C, these extended opening channels may be configured in arrays in a 2 dimensional or 3 dimension configuration, which presently known pore based sequencing systems cannot achieve.

[92] Referring now to Figure 16A, a serial probe array 1677 is shown. The probe array includes Q serial probe sets 1630. In general, extended objects to be analyzed may be passed through the Q serial probe sets 1630. The Q serial probe sets may be homogeneous or heterogeneous.

[93] For example, using homogeneous probe sets 1630, each probe set may include various individual probes optimized for adenine, cytosine, guanine, and thymine.

[94] Further, referring to Figure 16C, an array 1680 of probe sets may comprise heterogeneous probes. For example, one probe set may be optimized for adenine (A), a second optimized for cytosine (C), a third for guanine (G) and a fourth for thymine (T).

[95] These serial arrays would not be possible using conventional known techniques, for example, based on pores as described in the background of the invention. Importantly, redundancy is readily achievable in a serial configuration of the present invention, whether the system is formed of serial heterogeneous probe sets, serial homogeneous probe sets, or combinations thereof.

[96] Referring now to Figure 16B, a parallel and serial probe array 1678 is shown. The probe array includes M x N channels of Q serial probe sets 1630. This probe array 1678 may be very useful for high speed parallel processing of extended objects to be analyzed. The probe sets 1630 within the array 1678 may be homogeneous or heterogeneous. The extended objects may be the same or different. In general, extended objects to be analyzed may be passed through the Q serial probe sets. An M x N array of extended objects, which may be the same or different, are passed through the M x N arrays of Q serial probe sets 1630.

[97] The above described probes may be used in various configurations. Certain probes may be in the form of open tip probes. The various open tip probes described herein may be used for dispending materials, for example, as a nano-nozzle or

nano-funnel. Further, various open tip probes described herein may be used to expose a specimen or a workpiece to photonic energy or stimuli, serve as a as a nano-nozzle or nano-funnel for ion or particle beam operations, or the like.

[98] Further, various open tip probes described herein may be used to expose materials to a specimen or a workpiece, whereby a) forces are applied within the body of the probe, within the well of the probe, or by another element within the probe to keep the material from dispensing; b) operate at suitable temperature the reduces the likelihood of or prevents the material from dispensing; or c) operate at suitable pressure the reduces the likelihood of or prevents the material from dispensing). ,

[99] Certain probes may be in the form of nano-electrodes for measuring detectable interactions. Certain probes may be in the form of materials that result in detectable interactions such as a system of correlating biological materials that create hybridization events with the extended object to be analyzed.

[100] In certain embodiments, and referring now to Figures 17A and 17B, the basic principle is described, wherein a DNA chain (or other protein or extended object to be analyzed) 1750 upon a base 1728 is passed underneath four probes in the open tip probes 1742, 1744, 1746 and 1748 (or arrays of nozzles, e.g., as shown in Figure 17B). The four funnels or nozzles 1742, 1744, 1746 and 1748 are filled with adenine, cytosine, guanine, and thymine molecules respectively. Due to the complementary structures of adenine and thymine, and of guanine and cytosine, a hybridization event between nucleotides on the DNA chain and the nucleotides in the nozzle will occur when the correct pairs come into contact. This hybridization results in a lower energy state and charge transfer, which can be detected via an ammeter. This is because the conductivity

between the nozzles and the electrode ground plate will be affected, thereby altering the current between the nozzle and the ground plate. Figure 17B shows an exemplary array setup, e.g., that may average out noise and increase SNR. These features will help in assuring an excellent SNR.

[101] Note that the above described probes may also be formed with one or more conductors therein for increase signal detection capabilities. For example, the conductor may be layered within or upon an inner wall of the probe or nozzle well and tip/

[102] Referring to Figure 18A, an embodiment of a system 1800 having probes formed of solid state nucleotide materials is shown. A probe set 1830 is depicted wherein each probe 1842, 1844, 1846, 1848 is formed of a solid state nucleotide, e.g., adenine, cytosine, guanine, and thymine molecules respectively. A solid state nucleotide may be manufactured on thin films, and formed as probes using the various manufacturing methods described herein or other thin film manufacturing techniques. Preferably, these SSN have a single molecule thickness at the probe tip, so that a desirable monomer scale resolution is maintained. These films may be formed in the nozzle wells, e.g., by layering during the manufacturing process prior to slicing. In preferred embodiments of a DNA sequencing system herein, the nozzles are formed with a tip dimension of less than about 0.5 nanometers to resolve corresponding monomers.

[103] It is known that DNA strands may be condensed on substrates. In the herein probes, single species nucleotide strands may be condensed in the form of lines or films. Referring to Figure 18B, these may be formed on a substrate (M), such as a

conductive substrate, Referring to Figure 18C, condensed single species nucleotide strands may be sandwiched between substrates (M).

[104] The films resulting from Figures 18B or 18C may be used directly as the probes. Alternatively, these films may be slices and attached to metallic "knife blades". In a further alternatively, they may be folded, whereby exposed condensed single species nucleotides serve as the probe.

[105] Referring now to Figure 19, a system 1900 is shown using a metal conductors as probes 1931. The probe may be formed of a suitable conductor material. Further, probes in the form of nozzles may be filled or layered with metal conductor material. The metal may be platinum, gold, or other suitable metal or non metal conductor. In preferred embodiments of a DNA sequencing system herein, the conductor probes formed to a tip dimension of less than about 0.5 nanometers to resolve corresponding monomers.

[106] In one method of using a probe 1931, stimuli (e.g., a voltage) is applied across the subject nucleotide within the subject strand, and a characteristic I vs. V curve may be obtained. For example, Figure 20 shows an exemplary representation of characteristic curves for various monomers adenine, cytosine, guanine, and thymine (A, C, G and T).

[107] In certain embodiments, a single probe 1931 may be used as described in Figure 19. In other embodiments, a probe set may be used, whereby bias waveforms across different electrodes may be varied to adjust sensitivity for expected specimen portions or monomers. For example, a four-probe probe set may be used for identifying A,C,T,G components of biopolymers such as DNA strands. Further, identical

waveforms may be applied whereby multiple probes are used for redundancy. These may be gated or un-gated, depending on the application.

[108] Referring now to Figure 21, a functionalized group 2150 (FG) is mounted onto probe 2110. The 2150 may include known nucleotide strands, oligomers, peptides, single molecules, or other known species. The 2150 is selected to have a known specific sensing capability, for example, electrostatic, magneto-static, chemical, and other interactions with a specimen under analysis.

[109] Referring now to Figure 22, a functionalized group 2250 may be attached to cylinders of micrometer diameters which may then be attached to a larger structures. The cylinders may be coated glass, metal, or organic or inorganic.

[110] Referring now to Figure 23, plural functionalized groups 2352, 2354, 2356 are is mounted onto a probe 2310. In this embodiment, stepping operations, either of the probe or the specimen, is in two directions. By stepping in a direction substantially normal to the width *w* of the probe 2310 and in a direction substantially parallel to the width *w* of the probe 2310, analysis may be simplified. For example, functionalized group 2352 interacts with the specimen, the observation is recorded, then the probe is stepped so that functionalized group 2354 interacts with the specimen, the observation is recorded, and then the probe is stepped so that functionalized group 2356 interacts with the specimen, the observation is recorded. Then, the entire probe may be stepped in a direction substantially normal to the width *w* of the probe 2310 to continue analyzing the specimen.

[111] Referring now to Figure 24A, an embodiment of a system 2400 having probes formed of conductor with a known material strand attached to the edge of

the probe, particularly the "knife edge" probe, e.g., described above with respect to Figures 2 and 3. For example, a probe set 2430 is depicted wherein each probe 2442, 2444, 2446, 2448 has a known nucleotide strand, e.g., adenine strand, cytosine strand, guanine strand, and thymine strand respectively.

[112] In a preferred embodiment, a single strand/single species nucleotide strand is provided. It is stretched and attached to the tip of a conductor probe.

[113] The known nucleotide strand may be attached to the tip if the conductor probe by various nano- or micro- manipulation means.

[114] In one embodiment, magnetically attractive molecules, referred to as "magnetic beads", may be attached at opposing ends of the known strand to facilitate manipulation. A nano-manipulator magnet system may be used to stretch the strands for attachment to the probe set. For example, this is shown with respect to Figure 24B. Further, this configuration ensure that as the probe passes over the specimen, landing error associated with typical probe analysis systems is eliminated.

[115] With a single-strand, single-species chain attached at the probe tip, when the tip encounters a specimen portion or monomer that is capable of forming a hybrid pair with the probe species, bond energies associated with the hybridization event enhances the resonance activity being measuring.

[116] Referring to Figure 25, an embodiment of a system 2500 having probes formed as open wells or funnels is shown. A probe set 2530 is depicted wherein each probe 2542, 2544, 2546, 2548 is formed as an open well or funnel. This open well or funnel may be used as a path for various probe activities, for example, generated by sources 2582, 2584, 2586, 2588.

[117] Particle beam emitters can be made directly into nano probes or indirectly through the funnel described herein. They include ion beam and electron beam emitters.

[118] Photon beam emitters such as x-ray emitters, ultraviolet emitters, IR emitters, visible emitters, and terahertz emitters can be formed with the herein probes or trough funnels as described herein. In the event that the excitation photon beams have wavelengths large than the probe diameter, the use of evanescent fields that extend only to the width scale of the beam (probe) will be utilized.

[119] In another embodiment, an electron beam emitter is focused and shaped to provide a nano-scale resolution beam. They can be tuned in energy. This tunability can give one selectivity in directly interacting with the specimen to be analyzed. Electron beams may be used as the probe for the systems of the present invention.

[120] It is known in the electron optics art that atomic scale resolution may be achieved with SEM, TEM, and STEM since the beams themselves can be made nano-scale as the probing beams. In preferred embodiments of a DNA sequencing system herein, the electron beams are focused to a sectional dimension of less than about 0.5 nanometers to resolve corresponding monomers. The electron beam may be a line beam (analogous the probe of Figure 2), or electron beam scanning may be employed (analogous the probe of Figure 3, although it is to be understood that the funnel need not be moved, only the beam).

[121] Referring to Figure 25, the electron beam may be inserted through the funnel. This minimized the need for nano-scale resolution electron optics required for direct electron beam formation at the atomic scale.

[122] It should be appreciated that the funnel walls for x-ray, electron beams and ion beams will be constructed appropriately to be able to propagate from the funnel opening to the funnel end to achieve nano-scale resolution. In the case of electron beams, electric fields appropriately placed may cause these beams to bend toward the funnel tip. Alternately, secondary electron emission may be created from inner funnel wall surfaces which lead to the creation of a beam that exits the funnel tip.

[123] In another embodiment, a focused ion beam emitter with nano-scale resolution known in the art may be used as the probe to interact with the specimen. They can be tuned in energy. This tunability can give one selectivity in directly interacting with the specimen to be analyzed. Further, the ion beams may be based on H+, He+, Ge+, Ga+, or other suitable ions of substances that may be formed into beams that have specific selective interaction with the specimen to be resolved.

[124] Referring to Figure 25, the ion beam may be inserted through the funnel. This minimized the need for nano-scale resolution electron optics required for direct electron beam formation at the atomic scale.

[125] It should be appreciated that the funnel walls for x-ray, electron beams and ion beams will be constructed appropriately to be able to propagate from the funnel opening to the funnel end to achieve nano-scale resolution. In the case of electron beams, electric fields appropriately placed may cause these beams to bend toward the

funnel tip. Alternately, secondary electron emission may be created from inner funnel wall surfaces which lead to the creation of a beam that exits the funnel tip.

[126] X-ray beams, such as an x-ray laser beam, may be used as the probe for the systems of the present invention. In preferred embodiments of a DNA sequencing system herein, the x-ray beams are focused to a sectional dimension of less than about 0.5 nanometers to resolve corresponding monomers. For example, the electron beam system described above may be used to generate nano-scale x-ray beams in a manner known in the art.

[127] Further, referring to Figure 25, an x-ray beam (directly or indirectly) may be inserted through the funnel. This minimized the need for nano-scale resolution x-ray and electron optics required for direct electron beam formation at the atomic scale.

[128] It should be appreciated that the funnel walls for x-ray, electron beams and ion beams will be constructed appropriately to be able to propagate from the funnel opening to the funnel end to achieve nano-scale resolution. In the case of x-ray, the inner surfaces of the funnel may be made of multi-surface to achieve interference reflection, or may be of single crystal using Bragg reflection properties, or may be grazing incidence angle rejection until the rays reach the funnel end.

[129] To avoid stray x-rays that may interfere with excitation and/or measureant and increase noise, the inner and outers surfaces of the funnel as appropriate may be coated with x-ray absorbers.

[130] Scanning tunneling microscopy(STM) or atomic force microscopy(AFM) probe tips may be arranged into arrays and utilized according to the teachings of the present invention.

[131] The above described probes may be used in various configurations. Certain probes may be in the form of wells with dispending tips. Certain probes may be in the form of nano-nozzles. Certain probes may be in the form of nano-funnels. Certain probes may be in the form of electrodes for lithography.

[132] As described herein, for example, with respect to Figures 10 and 11, probes may be provided herein with variable dimensioned or actuate-able tip openings. This type of variable gap probe may be very useful for many applications, including but not limited to controlled dispensation of materials, controlled vacuum or fluid pressure, manipulation of nanometer sized structures, and other applications.

[133] Various configurations of the open tip probes herein may be useful for vacuum or fluid pressure. For example, certain embodiments of the open tip probes described herein may be used to impart vacuum or fluid pressure. In another embodiment, and referring now to Figure 26, a probe 2610 is provided having a plurality of openings 2612 along the length of the extended width probe tip, with other regions 2614 plugged with suitable plug material. The vacuum or fluid source may further be divided, or alternatively, the plural openings 2612 may share a common vacuum or fluid source.

[134] Herein disclosed are probes, nano-probes and methods of manufacturing probes and nano-probes. With the disclosed methods, it is possible to create probes with tip active area dimension, such as opening dimensions in the cases where the probe has an open tip, on the order of about 0.1 nanometers to about 10 nanometers, 10 nanometers to about 100 nanometers, or 100 nanometers to 1000 nanometers. Further, it is possible to make such probes in arrays with exact spacing

therebetween, and with additional supporting functionality such as stimuli providing structures, metrology structures, micro- and nano-fluidic structures or devices, micro- and nano-electromechanical structures, or other supporting features. Such features enable molecular level dispersion, precise material deposition, molecular level detection, and other nano-scale processes.

[135] Furthermore, the herein described analytical systems including sequencing of extended objects such as DNA or RNA strands or fragments is enabled by creating a probe having tip dimensions on the order of about 5 Angstroms, for example, utilizing the herein referenced and described probe and nozzle manufacturing methods. There are various methods of making the probes, probe sets and probe arrays described herein. Co-pending U.S. Non-provisional Application Serial No. 10/775,999 filed on February 10, 2004 (and corresponding PCT Application PCT/US04/03770) entitled "Micro-Nozzle, Nano Nozzle and Manufacturing Methods Therefor", incorporated herein by reference, describe various techniques for manufacturing probes in the form of nozzles or funnels are described. These techniques may be modified to provide other probe configurations and probe types described herein.

[136] Further, in certain embodiments, it may be desirable to conduct various fabrication, handling and assembly steps in clean room environments. In other embodiments, it may be desirable to conduct various fabrication, handling and assembly steps in a negative pressure environment and/or in ultra-pure inert gas environments.

[137] In general, in certain embodiments of the herein described methods of making the films, the probe tip active area has relevant tip dimensions (e.g., tip width t as shown in the above Figure 2A) that is a function of a very thin film that is layered,

deposited, or otherwise formed either on a portion of a probe body or on intermediate structures between plural probes.

[138] Prior art teaches how sub-micron objects and features can be produced by means of conventional optical, UV, e-beam, X-ray and lithography. These tools are being extended to produce sizes below 30 nanometers. As they are stretched to produce even smaller sizes, their limitations become more and more apparent, in terms of cost, foot-print, etc. Indeed, at high electron and ion beam accelerating voltages > 100KV features smaller that 10 nm have been demonstrated. The preparation steps and the cost of the equipment and ancillary components make these prior art methods cumbersome and slow.

[139] The present invention, shows ways to produce similar or better results faster, and more convenient by departing from using lithography based photon, ion and e-beams to produce the smallest features. Instead, ultra-thin films are used for this purpose.

[140] There are many known methods of producing films with atomic precision. These include, deposition by sputtering, electron beam, ion beam, molecular beam epitaxy, CVD, MOCVD, plasma, laser deposition, pyrolitic deposition, electrochemical, thermal evaporation, sputtering, electro-deposition, molecular beam epitaxy, adsorption from solution, Langmuir-Blodgett (LB) technique, self-assembly and many other related methods collectively referred to as Thin Film Deposition Methods. Accurate metrology enables the production and control of thicknesses with Angstrom precision. Producing free standing films by peeling is possible as taught in copending U.S. Patent Application Serial No. 09/950,909 filed on 9/12/2001 and U.S. Patent

Application Serial No. 10/970,814 filed on October 21, 2004 and manipulation taught in applicant's co-pending U.S. Non-provisional Application Serial Nos. 10/717,220 filed on November 19, 2003 entitled "Method of Fabricating Muti Layer Mems and Microfluidic Devices" and other related applications. The films produced by the conventional deposition methods need atomically flat substrates.

[141] The advent of scanning tunneling microscopy (STM), atomic force microscopy, AFM, scanning probe microscopy, SPM, and related tools have enabled the imaging of surfaces and structures with atomic resolution. This opened new vistas to advance our understanding of many physical and chemical phenomena that are being exploited in numerous practical applications in the fields of medicine, nanotechnology, nano-electronics, genomics, proteomics, nano-electrochemistry, and destined to make even more contributions in other fields in the futures.

[142] To achieve nano-scale resolution and nanofabrication accuracy and to properly interpret physical and chemical phenomena, it necessary to use atomically flat, atomically smooth substrates over a large area preferably in the range of several square microns to several square centimeters . To produce such substrates, prior art relies of unsophisticated and inaccurate techniques of attaching an adhesive tape to the surface of mica or graphite to peel the top most atomic layers to reveal a fresh atomically smooth surface of a piece of mica or graphite of size and tetchiness. In almost all situations the atomic surface is the desired result while the lateral shape or size or thickness is of little importance. Prior art techniques could not teach methods of producing , handling and manipulating samples having a single layer graphite (also called graphene) or mica of a predetermined desired number of mono-atomic of mica or graphite.

[143] Graphites are well known and are widely used materials. For example US 6,538,892 exploits its good mechanical and anisotropic thermal properties for the construction of heat sinks. Graphites according to the description in US 6,538,892, are made up of layer planes of hexagonal arrays or networks of carbon atoms. These layer planes of hexagonally arranged carbon atoms are substantially flat and are oriented or ordered so as to be substantially parallel and equidistant to one another, as shown in Figure 27. The substantially flat, parallel equidistant sheets or layers of carbon atoms, 2710, usually referred to as graphene layers or basal planes, are linked or bonded together and groups thereof are arranged in crystallites. Highly ordered graphites consist of crystallites of considerable size: the crystallites being highly aligned or oriented with respect to each other and having well ordered carbon layers. In other words, highly ordered graphites have a high degree of preferred crystallite orientation. It should be noted that graphites possess anisotropic structures and thus exhibit or possess many properties that are highly directional e.g. thermal and electrical conductivity and fluid diffusion.

[144] Briefly, graphites may be characterized as laminated structures of carbon, that is, structures consisting of superposed layers of carbon atoms joined together by weak van der Waals forces 2712. In considering the graphite structure, two axes or directions are usually noted, to wit, the "c" axis or direction and the "a" axes or directions. For simplicity, the "c" axis or direction may be considered as the direction perpendicular to the carbon layers. The "a" axes or directions may be considered as the directions parallel to the carbon layers or the directions perpendicular to the "c" direction.

The graphites suitable for manufacturing flexible graphite sheets possess a very high degree of orientation.

[145] The bonding forces holding the parallel layers of carbon atoms together are only weak van der Waals forces. In a process referred to as exfoliation of graphite, natural graphites can be treated so that the spacing, d, in Fig. 27A between the superposed carbon layers can be appreciably opened up so as to provide a marked expansion of Nd ,as in Fig. 27B, the direction perpendicular to the layers, that is, in the "c" direction, and thus forms an expanded graphite structure in which the laminar character of the carbon layers is substantially retained. It has-been shown that N can be in the range of 100 to 1000 according to the treatment process. The graphite layers are referred to as graphene layers possess very high electrical and thermal conductivities exceeding those of copper, while retain high temperatures and exceedingly Young modulus.

[146] Recently, Andrei Geim and colleagues of the University of Manchester isolated a single sheet of graphene and measured its remarkable properties which include conductivity 100 higher than copper and astonishing Quantum Hall Effect behavior. These and other results are described in January, 2006 , Physics Today. These results could be made possible only after successful isolation of a single 1 Angstrom graphene layer, a feat that was not previously possible. Geim's team succeeded in isolating a single graphene layer by random and tedious and unpredictable method. According to the Physics Today Article : "Their method is astonishingly simple: Use adhesive tape to peel off weakly bound layers from a graphite crystal and then gently rub those fresh layers against an oxidized silicon surface. The trick was to find the relatively

rare monolayer flakes among the macroscopic shavings. Although the flakes are transparent under an optical microscope, the different thicknesses leave telltale interference patterns on the SiO2, much like colored fringes on an oily puddle. The patterns told the researchers where to hunt for single monolayers using atomic force microscopy.

[147] The work confirmed that graphene is remarkable—stable, chemically inert, and crystalline under ambient conditions."

[148] From the above and other recent investigations on graphene as well as from commercial supplier of graphite substrate, one concludes that there is a need for inventing convenient, low cost, and fast methods for isolating single layers of graphene and predictable stacks of selected number of graphene layers. There is further the need for general methods for isolating single layer or predictable number of layers from lamellar or multilayer materials which include but not limited to mica, Super lattices MoS2, NbSe2, Bi2Sr2CaCu2Ox, graphite, mica, Boron nitride , dichalcogenides, trichalcogenides, tetrachalcogenides, pentachalcogenides and Hydrotalcite-like materials.

[149] Therefore, many aspects of the present invention involve production of single and multiple layers of lamellar material. Many of the inventive features and certain embodiments of the present invention rely on the ability to make ultra-thin, nano-scale films. In further embodiments, it is desirable that these films are are atomically flat films. These enable the fabrication of all the probe configurations that perform a variety of functions necessary to advance the frontier of nano-science and technology including but not limited to imaging, analysis, sequencing, nano-lithography, and nano-manipulation as well a variety of other applications. Thin film deposition methods

describe above may be used to produce thing films with Angstrom precision. Alternatively, even more precisely define thickness can be produced the controlled peeling of one or more predetermined number of layers from lamellar material as taught herein. These embodiments described herein apply to graphite to produce graphene layers, to producing layers of mica, MoS2 and lamellar materials.

[150] One embodiment to selectively peel off a single layer from a lamellar material , 2810, is illustrated in Figure 28. The material is cut along the line 2812, at an angle of, for example, 20 degrees or more relative the "c" axis. The goal is the have access to the top most layer 2822, as each layer is sequentially removed according to Figure 28B. Two knife edge probes, as described herein, having tip opening dimensions small enough to access individual layers or groups of layers that are revealed due to the angular cut, are use to facilitate the peeling process. Knife edge probe 2818 pushes down on the second layer against the first substrate 2814 while knife edge probe 2820, pushes up the first layer against a second substrate 2816, attached to the desirable first layer. Figure 28C shows the complete separation of the first layer that is attached to the substrate 2816 which is being pulled vertically to facilitate the separation process.

[151] In another embodiment, the knife edges 2818, 2820, are applied in the horizontal directions pushing on both sides pry loose the first layer while the substrate 2816 is pulling upward. This method illustrated in Figures 28D-F, is facilitated with the knowledge of the exact separation between layers by known imaging techniques such as AFM and STM. This information, along with well know tools to move the knife edges with sub-angstrom precision, allows for reliable separation of the layers.

[152] Figure 29 illustrates yet another embodiment to reliably separate single layers. It exploits etching the peripheral regions of the first layer to expose the second layer by known etching techniques including electrochemical etching as shown in Figure 29A. Here, a voltage source 2918 is applied actoss electrodes 2915, that are contacting the peripheral regions 2910, of the first layer 2922.

[153] The exposed second layer 2912 is pushed as in Figure 29B. After the etching is complete, the electrodes 2915 push down on the second layer against the substrate 2914 while the top substrate 2916 is pulling upward the selected first layer 2922. Thus a single layer is conveniently and inexpensively removed and transferred to a third substrate, optionally. The substrate 2916 is removably bonded to the first layer 2922 by many bonding techniques including but not limited to adhesives, waxes, vacuum, etc. The final result in 29C is repeated for all the other layers of the lamellar material until all layers are removed with minimum of waste. This method can also be combined with method described in Figure 28A-F above to allow for the selection and the removal of more than a single layer. For instance, in the graphene case, it may be desirable to have a single layer of 1 Ang, 2 layers of 2 Ang, N layers of multiple Ang, depending on how the graphite is exfoliated to swell the interlayer spacing by factors of 10-1,000. (See exfoliated graphite description presented above with respect to Figures 27 A and 27 B).

[154] Another embodiment that takes advantage of the unique properties of graphene and metallically coated other lamellar materials is described in Figure 30. A special substrate 3016 is provided and is removable attached to the first layer 3022 we intend to peel. Current source 3012 is applied to the first graphene layer 3022 and

electrode 3024 deposited on top of substrate 3016. The current 3028 flowing in electrode 3024 and flowing out (in the opposite direction) of single layer 3022 result in a magnetic force 3020 that selectively pulls upward in the upward direction 3018, only the first layer 3022. By further applying a mechanical force upward to substrate 3016, the combination of magnetic and mechanical forces allows peeling with ease 3022. Since no such forces are influencing second and third layers, they are left intact. Separation process is illustrated in 30A-B.

[155] Instead of exploiting the magnetic force in the aforementioned embodiment, it is possible to use instead electrostatic force ad illustrated in 31A-B. In this case a voltage source 3116 is applied to electrode 3124, deposited on substrate 3112 and a revealed portion of the first layer 3122. The electric field 3120 is applied and causes an electrostatic force in the upward direction 3118, and along with a mechanical force applied to a substrate upward in a pulling selection, the first layer is selectively removed from the entire multi layer structure 3110.

[156] Another embodiment of peeling layers of lamellar material is shown in Figures 32A-C. Here the multilayer lamellar structure 3210 is attached to a substrate 3214 to the bottom while at the top implement substrate 3212 is removably attached to the top of the specimen. Said substrate 3212 may be a vacuum handler, adhesive tapes or other films with removable adhesives. The first step is to lift substrate 3212 which will pull or peel a random number of layers 3216, shown in Figure 32A. This process is repeated as necessary until the last few layers remain as in Figure 32B. In Figure 32C the second to last layer is finally removed, leaving the last layer 3222 bonded to substrate 3214. Note that the shavings, or the peelings of random number of layers are in turn

attached to substrate 3214 and the process is repeated until the desired number of single layers are removed and utilized.

[157] The above embodiments of methods to selectively remove single layers, or predetermined number of layers from lamellar could be combined as appropriate to achieve most advantageous, practical and economical way to produce the desired results.

[158] As discussed herein, in certain embodiments of the herein described methods of making the films, the probe tip active area has relevant tip dimensions (e.g., tip width *t* as shown in the above Figure 2A) that is a function of a very thin film that is layered, deposited, or otherwise formed either on a portion of a probe body or on intermediate structures between plural probes.

[159] As discussed herein, in certain embodiments of the herein described methods of making the films, the probe tip active area has relevant tip dimensions (e.g., tip width *t* as shown in the above Figure 2A) that is a function of a very thin film that is layered, deposited, or otherwise formed either on a portion of a probe body or on intermediate structures between plural probes.

[160] Using various film processing techniques invented by the inventor hereof and incorporated by reference herein above and below, ultra thin layers of materials are deposited to form a stack of layers. The probes areas may be formed as openings, whereby a series of probes may be readily formed by creating a stack of layers alternating between insulator or semiconductor materials and selectively removable materials, whereby the geometry and dimensions of the selectively removable materials defines the opening geometry and dimensions. Note the selectively removable materials

may also be placed adjacent a conductor, or between a pair of conductors, to, e.g., allow for controllable dispensing or other functionality.

[161] In other embodiments, the probes areas may be a suitable conductors, whereby a series of probes may be readily formed by creating a stack of layers alternating between insulator or semiconductor materials and conductive material, whereby the geometry and dimensions of the conductive material defines the probe or electrode geometry and dimensions.

[162] Certain methods to make the probes, probe sets, and probe arrays may utilize the processing techniques and various tools invented by applicants hereof suitable for processing thin layers and forming vertically integrated devices. Various probes and configurations thereof may be manufactured with the use of Applicant's multi-layered manufacturing methods, as described in U.S. Non-provisional Application Serial Nos. 09/950,909, filed September 12, 2001 entitled "Thin films and Production Methods Thereof"; 10/222,439, filed August 15, 2002 entitled "MEMs And Method Of Manufacturing MEMs"; 10/017,186 filed December 7, 2001 entitled "Device And Method For Handling Fragile Objects, And Manufacturing Method Thereof"; PCT Application Serial No. PCT/US03/37304 filed November 20, 2003 and entitled "Three Dimensional Device Assembly and Production Methods Thereof"; U.S. Patent No. 6,857,671 granted on April 5, 2005 entitled "Method of Fabricating Vertical Integrated Circuits"; U.S. Non-provisional Application Serial Nos. 10/717,220 filed on November 19, 2003 entitled "Method of Fabricating Muti Layer MEMs and Microfluidic Devices"; 10/719,666 filed on November 20, 2003 entitled "Method and System for Increasing Yield of Vertically Integrated Devices"; 10/719,663 filed on November 20, 2003 entitled

"Method of Fabricating Muti Layer Devices on Buried Oxide Layer Substrates"; all of which are incorporated by reference herein. However, other types of semiconductor and/or thin film processing may be employed.

[163] Referring now generally to Figures 33A-33F, a method and system for making a thin device layer 3320 that may be used as a probe or probe precursor, or may be used as a substrate for a probe, probe precursor, probe set, or probe array thereon or therein (generally referred to herein as "probe elements") according to various embodiments of the present invention. Figure 33A shows a bulk substrate 3302 as a starting material for the methods and structures of the present invention. Referring to Figure 33B, a release inducing layer 3318 is created at a top surface of the bulk substrate 3302. This release inducing layer 3318 may include a porous layer or plural porous layers. The release inducing layer 3318 may be formed by treating a major surface of the bulk substrate 3302 to form one or more porous layers 3318. Alternatively, the release inducing layer 3318 in the form of a porous layer or plural porous layers may be derived from transfer of a strained layer to the bulk substrate 3302.

[164] Further, the release inducing layer 3318 may include a strained layer with a suitable lattice mismatch that is close enough to allow growth yet adds strain at the interface. For example, for a single crystalline silicon substrate 3302, the release inducing layer in the form of a strained layer may include silicon germanium[1], other group III-V compounds, InGaAs, InAl, indium phosphides, or other lattice mismatched material that provides for a lattice mismatch that is close enough to allow growth, in

[1] For example, U.S. Patent No. 6,790,747 to Silicon Genesis Corporation, incorporated by reference herein, teaches using a silicon alloy such as silicon germanium or silicon germanium carbon, in the context of forming SOI; S.O.I.Tec Silicon on Insulator Technologies S.A. U.S.

embodiments where single crystalline material such as silicon is grown as the deice layer 3320, and also provide for enough of a mismatch to facilitate release while minimizing or eliminating damage to probes or probe precursors formed in or upon the device layer 3320. The release inducing layer 3318 may be formed by treating (e.g., chemical vapor deposition, physical vapor deposition, molecular beam epitaxy plating, and other techniques, which include any combination of these) a major surface of the bulk substrate 3302 with suitable materials to form a strained layer 3318 with a lattice mismatch to the device layer 3320 (e.g., silicon germanium when the device layer 3320 and the substrate 3302 are formed of single crystalline Si). One key feature of the release layer, particularly in the form of the strained layer, is that at least a portion of the release layer comprises a crystalline structure that is lattice mismatched compared to the bulk substrate and the device layer to be formed or stacked atop the release layer. Alternatively, the release inducing layer 3318 in the form of a strained layer may be derived from transfer of a strained layer to the bulk substrate 3302.

[165] In other preferred embodiments, the release inducing layer comprises a layer having regions of weak bonding and strong bonding (as described in detail in Applicant's copending U.S. Patent Application Serial No. 09/950,909 filed on 9/12/2001 and U.S. Patent Application Serial No. 10/970,814 filed on October 21, 2004, both entitled "Thin films and Production Methods Thereof" incorporated by reference herein, and further referenced herein as "the '909 and '814 applications").

[166] Still further, the release inducing layer may include a layer having resonant absorbing material (i.e., that absorbs certain exciting frequencies) integrated

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Patent No. 6,953,736, incorporated by reference herein, discloses using a lattice mismatch to form a strained silicon-on-insulator structure with weak bonds at intended cleave sites.

therein. For example, when certain exciting frequencies are impinged on the material such as during debonding operations, resonant forces cause localized controllable debonding by heating and melting of that material

[167] Referring to FIG. 33C, a device layer 3320 is formed on top of or within the release layer 3318. In certain preferred embodiments, the device layer 3320 is epitaxially grown, e.g., as an epitaxial single crystal silicon layer. In still further alternative embodiments, the device layer may be attached to the release layer and placed atop the substrate layer or bulk substrate 3302. For example, a suitable vacuum handler (such as one formed as described in 10/017,186 filed December 7, 2001 entitled "Device And Method For Handling Fragile Objects, And Manufacturing Method Thereof", incorporated by reference herein, or other vacuum handlers) may be used to hold and transfer a thin layer as mentioned above.

[168] A buried oxide layer may optionally be provided below the device layer 3320. For example, after the step described with respect to FIG. 33B, a portion of the release layer 3318 may be formed into an oxide layer or region. Alternatively, portions of the release layer 3318 may be treated to form buried oxide regions. Further, in another example, after the step described with respect to FIG. 33C, a portion of the release layer 3318 may be formed into an oxide layer or region, e.g., with suitable implantation treatment, or treated to form buried oxide regions. In a further alternative, where the device layer is attached to the release layer, the surface of the device layer intermediate the release layer may be treated to form an oxide layer, or an oxide layer may be deposited on the surface of the device layer intermediate the release layer.

[169] Referring to FIG. 33D, one or more probes and/or probe precursors 3322 may be formed in or upon the device layer. In certain embodiments, the device layer has wafer scale dimensions, whereby plural probes and/or probe precursors are formed on the wafer. The release layer 3318 allows the device layer 3320 to be sufficiently bonded to the bulk substrate 3302 such that during processing of the probes and/or probe precursors 3322, overall structural stability remains.

[170] Referring now to FIG. 33F, the device layer 3320 having probes and/or probe precursors 3322 thereon or therein may easily be separated from the bulk substrate 3302. As shown in Figure 33G, the device layer may optionally include a portion 3318' of the release layer. This may be kept with the device layer, or removed by conventional methods such as selective etching or grinding. This allows one to have a very thin device layer that may be used alone, e.g., for probes according to certain embodiments hereof. Alternatively, the thin device layer may be stacked to form a probe (e.g., in the case where the probe precursor is a portion of a probe that is stacked with another probe precursor, for example, stacked halves of a probe), or to form an array of probes. Further, the remaining substrate 3302 (which may have a portion 3318'' of the release layer) remains behind, which may be recycled and reused in the same or similar process after any necessary polishing.

[171] Accordingly, a method to make thin device layer utilizing the release layer described above with respect to Figs 33A-33F includes providing a structure A with 3 layers 1A, 2A, 3A, wherein layer 1A is a device layer, layer 2A is a release layer, and layer 3A is a support layer. In this manner, layer 1A is releasable from layer 3A. One or more probes and/or probe precursors are fabricated on the device layer 1A. Then, device

layer 1A may be released from support layer 3A. The support layer 3A may be reused for subsequent processes, e.g., as a support layer or as a device layer.

[172] As shown in Figures 33A-33F, release layer 3318 may comprise a layer of porous material, such as porous Si. In a further alternative embodiment, and referring now generally to Figures 34A-34G, a method and system for making a thin layer with a useful device thereon or therein is provided, wherein the release layer comprises a sub-layer 3418 of first porosity P1 and a sub-layer 3426 of second porosity P2. Thus, the release layer comprises a porous release layer having a sub-layer region of relatively large pores P1 proximate the substrate and a sub-layer region of relatively small pores P2 proximate the device layer. In certain embodiments, sub-layer region P1 is formed directly on said substrate. In other embodiments, sub-layer region P2 is grown on said sub-layer region P1. Note that although these representations show distinct sub-layers 3418 of first porosity P1 and sub-layers 3426 of second porosity P2, other porosity gradients across the thickness of the overall release layer may be used.

[173] Figure 34A shows a bulk substrate 3402 as a starting material for the methods and structures of the present invention. Referring to Figure 34B, a porous layer P1 (3418) is created at a top surface of the bulk substrate 3402.

[174] Referring to FIG. 34C, a second porous layer P2 (3426) may be formed on the first porous layer P1 (3418). In certain embodiments, a layer 3426 may be stacked and bonded to layer 3418. In certain other embodiments, a layer 3426 may be grown or deposited upon layer 3418.

[175] Referring to FIG. 34D, a device layer 3420 is formed on top of the porous layer P2 (3426). In certain embodiments, the device layer 3420 is epitaxially

grown, e.g., as a single crystal silicon layer. In still further alternative embodiments, the device layer may be attached to the release layer, e.g., transferred to the release layer.

[176] A buried oxide layer may optionally be provided below the device layer 3420. For example, after the step described with respect to FIG. 34B or 34C, a portion of the layer 3418 or 3426 may be formed into an oxide layer or region. Alternatively, portions of the layer 3418 or 3426 may be treated for form buried oxide regions. Further, in another example, after the step described with respect to FIG. 34D, a portion of the layer 3418 or 3426 may be formed into an oxide layer or region, e.g., with suitable implantation treatment, or portions of the layer 3418 or 3426 may be treated to form buried oxide regions. Alternatively, where the device layer is attached to the layer 3426, the surface of the device layer intermediate the release layer may be treated to form an oxide layer, or an oxide layer may be deposited on the surface of the device layer intermediate the release layer.

[177] Referring to FIG. 34E, one or more probes and/or probe precursors 3422 may be formed on the device layer. In certain embodiments, the device layer has wafer scale dimensions, whereby plural probes and/or probe precursors are formed on the wafer. The layer 3418 or 3426 allows the device layer 3420 to be sufficiently bonded to the bulk substrate 3402 such that during processing of the probes and/or probe precursors 3422, overall structural stability remains.

[178] Referring now to FIG. 34F, the device layer 3420 having probes and/or probe precursors 3422 thereon or therein may easily be separated from the bulk substrate 3402. As shown in Figure 34G, the device layer may optionally include a

portion 3426 of the porous layer P2. This may be kept with the device layer 3420, or removed by conventional methods such as selective etching or grinding.

[179] As shown in Figures 33A-33F and 34A-34G, release layer 3318 may comprise a layer of strained material, such as a layer of silicon-germanium (SiGe). For example, a layer of SiGe may be grown on a the substrate layer. Since germanium has a larger lattice constant than Si, the SiGe layer is compressively strained as it grows.

[180] Referring now to Figures 35A-35F, another method of making a thin layer including one or more probes and/or probe precursors therein or thereon is provided. A bulk substrate 3502 is provided (Figure 35A). Referring to Figure 35B, all or a portion of a surface 3504 of the bulk substrate 3502' is treated to form a region 3506. In this embodiment, as described below, region 3506 is formed of a material and/or having material characteristics to allow growth of a layer on top thereof, and also serve as a portion of the release layer, wherein portion 3506 represents a weak bond region as described above and described in further detail in Applicant's copending the '909 and '814 applications incorporated by reference herein. In the embodiment shown with respect to Figures 35A-35F, a portion of the surface 3504 of the bulk substrate 3502' is treated, whereby portions 3508 of the surface 3504 remain as the original bulk substrate which (shown in Figures 35B-35F as the periphery, but it is to be understood that other patterns may be created as described in Applicant's copending the '909 and '814 applications incorporated by reference herein). These portions represent strong bond regions as described in the '909 and '814 applications.

[181] Referring now to Figure 35C, a single crystalline material layer 3510 such as single crystalline silicon is epitaxially grown on top of the weak and strong

regions 3506, 3508. Figure 35D shows probes and/or probe precursors fabricated upon or within the single crystalline material layer 3510. Referring to Figure 35E, portions of the single crystalline material layer 3510 are removed corresponding to the regions of the portions 3508, and the portions 3508 are removed, for example by chemical etching, mechanical removal, hydrogen or helium implantation and heating of the portions 3508, or providing a material containing a resonant absorber at the portions 3508 for subsequent heating and melting of that material. Accordingly, a modified single crystalline material layer 3510' on the portion 3506 remains. Figure 35F shows the portion 3506 removed, thereby leaving single crystalline material layer 3510' with probe elements 3512 thereon or therein. Alternatively, single crystalline material layer 3510' with probe elements 3512 thereon or therein may be removed from the portion 3506, for example, by mechanical cleavage (parallel to the plane of the layers), peeling, or other suitable mechanical removal, whereby some residue of the portion 3506 may remain on the back of the single crystalline material layer 3510' with probe elements 3512 thereon or therein and some residue of the portion 3506 may remain on the top of the bulk substrate 3502'' left behind. In this manner, the bulk substrate 3502'' may be recycled and reused with minimal polishing and/or grinding, thereby minimizing waste of the single crystalline material of the bulk substrate 3502.. The single crystalline material layer 3510' with probe elements 3512 thereon or therein may be used as is, diced into individual devices or structures, or aligned and stacked (on a probe or probe array scale, or on a wafer scale) to form a probe, probe array, or plurality of probes and/or probe arrays.

[182] In certain embodiments, the strong bond portions 3508 may be formed by starting with a uniform layer. For example, the surface 3504 may comprise a strained material, such as silicon germanium. Utilizing zone melting and sweeping techniques, the germanium swept away from the desired strong bond regions 3508. When a layer 3510 is grown or formed on the layer having portions 3506, 3508, layer 3510 will be strongly bonded at the regions of portions 3508 and relatively weakly bonded at the regions of portions 3506.

[183] Referring now to Figures 36A-36F, another method of making a thin layer including one or more useful devices or structures therein or thereon is provided. A bulk substrate 3602 is provided (Figure 36A). Referring to Figure 36B, all or a portion of a surface 3604 of the bulk substrate 3602' is treated to form porous sub-regions 3605 and 3606. In this embodiment, as described below, region 3606 is formed of a material and/or having material characteristics to allow growth of a layer on top thereof, and also serve as a portion of the release layer, wherein porous sub-regions 3606/3605 represent a weak bond region as described above and described in further detail in the '909 and '814 applications incorporated by reference herein. In the embodiment shown with respect to Figures 36A-36F, a portion of the surface 3604 of the bulk substrate 3602' is treated (forming sub-regions 3605/3606), whereby portions 3608 of the surface 3604 remain as the original bulk substrate which (shown in Figures 36B-36F as the periphery, but it is to be understood that other patterns may be created as described in Applicant's copending the '909 and '814 applications incorporated by reference herein). These portions represent strong bond regions as described in the '909 and '814 applications.

[184] Thus, the release layer comprises sub-regions 3605/3606 and portions 3608. Sub-region 3605 has relatively large pores P1 proximate the substrate and sub- region 3606 has of relatively small pores P2 proximate the device layer to be described below. In certain embodiments, sub-region 3605 is formed directly on said substrate, and sub-region 3606 is grown on said sub-region 3605. In certain embodiments, sub-region 3606 may be stacked and bonded to sub-region 3605. In certain other embodiments, sub-region 3606 may be grown or deposited upon sub-region 3605.

[185] Referring now to Figure 36C, a single crystalline material layer 3610 such as single crystalline silicon is epitaxially grown on top of the weak and strong regions 3606, 3608. Figure 36D shows devices or structures fabricated upon or within the single crystalline material layer 3610. Referring to Figure 36E, portions of the single crystalline material layer 3610 are removed corresponding to the regions of the portions 3608, and the portions 3608 are removed, for example by chemical etching, mechanical removal, hydrogen or helium implantation and heating of the portions 3608, or providing a material containing a resonant absorber at the portions 3608 for subsequent heating and melting of that material. Accordingly, we are left with a modified single crystalline material layer 3610' on the portion 3606. Figure 36E shows an exemplary cleaving device, for example a knife edge device, water jet, or other device, used to cut between the sub-regions 3605 and 3606. Figure 36F shows the bottom portion of sub-region 3606 removed (with a portion of sub-region 3606 remaining on the bottom of the single crystalline material layer 3610), and the top portion of sub-region 3605 removed (with a portion of sub-region 3605 remaining on the bulk substrate 3602''). Accordingly, the

single crystalline material layer 3610' is left with devices or structures 3612 thereon or therein. In this manner, the bulk substrate 3602'' may be recycled and reused with minimal polishing and/or grinding, thereby minimizing waste of the single crystalline material of the bulk substrate 3602. The single crystalline material layer 3610' with devices or structures 3612 thereon or therein may be used as is, diced into individual devices or structures, or aligned and stacked (on a device or structure scale, or on a wafer scale) to form a vertically integrated device.

[186] Referring to Figure 37, a starting multiple layered substrate 3700 is shown. The substrate 3700 may be, in certain preferred embodiments, a wafer for processing thousands or even millions of probe elements, or be used to derive a very thin layers for use as probes and/or probe precursors.

[187] The multiple layered substrate 3700 includes a first device layer 3710 selectively bonded to a second substrate layer 3720, having strongly bonded regions 3703 and weakly bonded regions 3704. Using the techniques described in the above-mentioned patent applications, or other suitable wafer processing and handling techniques, the first layer 3710, intended for having one or more probe elements therein or therein, or used as a probe or probe precursor as a very thin layer, may readily be removed from the second substrate layer 3720 (which serves as mechanical support during device processing) with little or no damage to the structure(s) formed (including material deposited or otherwise incorporated, or wells or other subtractions to the layer 3710) in or on the device layer 3710.

[188] Accordingly, according to the methods of Figures 33 and 34, a layered structure is formed generally includes a first layer suitable for having a useful

element formed therein or thereon releasably attached or bonded to a second layer, e.g., a substrate. A method to form a layered structure generally comprises releasably adhering a first layer to a second layer. Further, according to the methods of Figures 35-37, a layered structure is formed generally includes a first layer suitable for having a useful element formed therein or thereon selectively attached or bonded to a second layer, e.g., a substrate, with regions of weak bonding and regions of strong bonding. The layered structure may be used for production of various devices including probes and/or probe precursors as provided for herein. Alternatively, a layered structure may be used as a source of one or more probes and/or probe precursors, for example, when the device layer is used as the probe, whereby the capability to produce and remove with little or no damage allows for ultra thin layers that may be used for ultra high resolution probes.

[189] The separation, for example, shown at steps of Figures 33E, 34F, 35E and 36E, may comprise various separation techniques. These separation techniques includes those described in further detail in Applicant's copending the '909 and '814 applications, incorporated by reference herein. The separation may be multi-step, for example, chemical etching parallel to the layers followed by knife edge separation. The separation step or steps may include mechanical separation techniques such as peeling, cleavage propagation; knife edge separation, water jet separation, ultrasound separation or other suitable mechanical separation techniques. Further, the separation step or steps may be by chemical techniques, such as chemical etching parallel to the layers; chemical etching normal to the layers; or other suitable chemical techniques. Still further, the separation step or steps may include ion implantation and expansion to cause layer separation.

[190] The material for the layers used herein, as the device layer, the release layer and the substrate layer, may be the same or different materials, and may include materials including, but not limited to, any of the lamellar materials described above, plastic (e.g., polycarbonate), metal, semiconductor, insulator, monocrystalline, amorphous, noncrystalline, biological (e.g., DNA based films) or a combination comprising at least one of the foregoing types of materials.

[191] Further, the release layer may comprise a material layer having certain amounts of dopants that excite at known resonances. When the resonance is excited, the material may locally be heated thereby melting the areas surrounding the dopants. This type of release layer may be used when processing a variety of materials, including organic materials and inorganic materials.

[192] The device layer and the substrate layer may be derived from various sources, including thin films described herein, wafers or fluid material deposited to form films and/or substrate structures. Where the starting material is in the form of a wafer, any conventional process may be used to derive the device layer and/or the substrate layer. For example, the substrate layer may consist of a wafer, and the device layer may comprise a portion of the same or different wafer. The portion of the wafer constituting the device layer may be derived from mechanical thinning (e.g., mechanical grinding, cutting, polishing; chemical-mechanical polishing; polish-stop; or combinations including at least one of the foregoing), cleavage propagation, ion implantation followed by mechanical separation (e.g., cleavage propagation, normal to the plane of the layers, parallel to the plane of the layers, in a peeling direction, or a combination thereof), ion implantation followed by heat, light, and/or pressure induced layer splitting), chemical

etching, or the like. Further, either or both the device layer and the substrate layer may be deposited or grown, for example by chemical vapor deposition, epitaxial growth methods, or the like.

[193] The dimensions of the device layers may also vary in thickness and surface area. For example, fabrication of probes having ultra high resolution may benefit from the methods and embodiments herein, whereby probes may be formed on layers that are a few tenths of a nanometer to a few nanometers.

[194] The surface areas for the methods and embodiments of the present invention may be die-scale, wafer scale, or in larger sheets; accordingly, surface areas may be on the order of nanometer(s) squared to a few microns squared for die-scale; on the order of a centimeters squared for wafer-scale; and on the order of centimeters squared to a meters squared for sheet scale.

[195] Referring now to Figures 38A and 38B, top isometric and sectional views, respectively, are provided of a selectively bonded substrate 3800 having a plurality of wells 3830 formed in the weakly bonded regions of the selectively bonded substrate 3800. The wells may be formed by etching, mechanical subtraction methods, ion or particle beam etching, or other suitable methods. Note that the pattern of weak bond regions and strong bond regions may vary, as described in the '909 and '814 applications. However, in certain preferred embodiments, all of the wells 3830 are formed at the weak bond regions of the device layer 3810 and supported during processing by the support layer 3820.

[196] Figures 38C and 38D show plan and sectional views, respectively, of a single well 3830 formed in the device layer 3810 described above. Referring to Figure

38C, the intersecting region between the dashed lines and the walls 3832 of the wells 3830 shows regions wherein probe elements may be processed in certain embodiments, as described hereinafter. In other embodiments, there may be only one intended region for processing nozzles (e.g., on the left or right sides as shown in Figures 38C and 38D).

[197] In further embodiments, the wells may be formed only at the intended probe element region, e.g., resembling grooves having the thickness shown by the dashed lines.

[198] Referring also to Figure 39, the well 3830 generally has angular walls 3832, the function of which will be readily apparent. Further, the center recessed portion 3834 of the well will become part of a reservoir of the probes. At the top surface of the device layer 3810 adjacent the outer ends of the angular walls 3832 are plateau regions, which ultimately may be part of the inside wall of the probes as described herein.

[199] Referring now to Figure 39, a layer 3810 (e.g., having thickness on the order of about 0.1 nanometers to about 10 nanometers, 10 nanometers to about 100 nanometers, or 100 nanometers to 1000 nanometers) is selectively bonded to a support layer 3820 as described with respect to Figures 33-37 and in the '909 and '814 applications. A region of reservoir 3830 is etched away or otherwise removed from a region of the device layer in the weak bond region 3803. Suitable nano-scale material subtraction methods may be used.

[200] Referring now to Figure 40A, a layer 3838 (e.g., having thickness on the order of about 0.1 nanometers to about 10 nanometers, 10 nanometers to about 100 nanometers, or 100 nanometers to 1000 nanometers) of material, preferably material that is easily removable by etching or other subtractive methods, is deposited on the wafer.

This material may be conductive, such as copper, silicon oxide, aluminum, or other suitable materials. This space will later become the opening for the nozzle.

[201] Referring to Figure 40B, a fill material 3840 may optionally be incorporated, also of easily removable material in certain embodiments. The material optionally used to fill the wells during processing and stacking may be the same or different from the material used at the plateaus (that will form nozzle walls).

[202] In certain embodiments, since the device layer including the etched well having suitable material deposited thereon is generally positioned over the weak bond region 3803 of the multiple layered substrate 3800, the device layer 3810 may readily be removed from the support layer 3820. For example, the strong bond regions 3804 may be etched away by through etching (e.g., normal to the surface through the thickness of the device layer in the vicinity of the strong bond region), edge etching (parallel to the surface of the layers), ion implantation (preferably with suitable masking of the etched well and deposited material to form the nozzle, or by selective ion implantation), or other known techniques. Since the above techniques are generally performed at the strong bond regions 3804 only, the etched well and material deposited in the weak bond regions 3803 are easily released form the substrate, as schematically shown in Figure 41, for example with a handler 3850.

[203] Referring now to Figure 42, several layers 3810 including etched wells 3830 having material deposited 3838 thereon (and optionally fill 3840) may be stacked to form a structure 3860. The structure 3860 may further include a solid layer 3862, e.g., to form a wall for the top-most nozzle as shown in Figure 42. Although in certain embodiments precise alignment may be desired at this point, certain embodiments

may use relaxed alignment standards at this point, as will be apparent from the further described steps.

[204] As shown in Figure 43, the wafer stack 3860 can now be sliced along a cut line 3864, creating two portions with exposed reservoirs. From the opposing side, these devices can also be sliced along the line 3866. The end may be grinded and polished until it is very close to the etched away reservoir, but no less than the desired nozzle length.

[205] Referring now to Figures 44 and 45, the deposited material 3838 may be etched away, exposing an etched channel 3868 (e.g., 5 nm opening when the material deposition layer is 5 nm). A material reservoir 3870 (or region 3870 for other purposes, depending on the desired use of the nozzle structure) remains behind the opening 3868. Each etched channel 3868 is generally spaced apart by approximately the thickness of the device layer 3810. Thus, a nozzle device 10 having plural openings 3868 each associated with regions 3870 is provided. Accordingly, when the thickness of the material to be removed is extremely small, e.g., on the order of about 0.1 nanometers to about 10 nanometers, 10 nanometers to about 100 nanometers, or 100 nanometers to 1000 nanometers, the extended edge probe tip as described above is created at the openings 3868.

[206] Alternatively, and referring to Figure 46, to form an opening less than the width of the entire edge, the outside portions may be masked 3872 prior to etching the deposited material 3838 to form openings 3868'. Thus, a nozzle device 3810' having plural openings 3868' is provided. Accordingly, the width (i.e., the y direction as shown in Figures 44-46) of the probes may be the same or different from the width of the

wells. In certain embodiments, it may be desirable to provide wells having widths larger than that of the nozzle to increase the material capacity of the well while maintaining the nozzle dimensions as small as possible.

[207] In a further embodiment, and referring now to Figures 47 and 48, a nozzle device 3880 (e.g., as describe herein), of a single layer, may be rotated approximately 90° with respect to the stack of layers 3860 having layers 3838 deposited therein at the locations of the nozzles. Etchant may be filled in the reservoir of the rotated nozzle structure 3880, and the openings 3882 of the nozzles may be formed. Using this technique, it is possible to create nozzles having approximately the same width and height with extremely small dimensions as provided for herein. Thus, a nozzle device 3810" having plural openings 3868" is provided.

[208] Referring now to Figures 49 and 50, another embodiment of a method of forming very small width nozzle diameters. As described with reference to Figures 44 and 45, the deposited material between layers may be etched away, exposing an etched channel spaced apart by approximately the thickness of the device layer.

[209] These etched channels 3868 may then be filled with an etchable material. For example, a nozzle device 3880 as describe herein, of a single layer, may be rotated approximately 90° with respect to the stack of layers having material etched away at the locations of the nozzles. An etchable material may be filled in the reservoir of the rotated nozzle structure, which is filled at the regions where the nozzles on the stack of layers are to be formed. The surrounding areas between the layers are then filled with a plug material. Then the etchable material in the nozzle region is etched away, exposing the nozzles 3868'''. Using this technique, it is possible to create nozzles having

approximately the same width and height of extremely small dimensions. Thus, a nozzle device 3810''' having plural openings 3868''' is provided.

[210] Note this etchable material should be selectively removable by an etchant (e.g., not removing the bulk material).

[211] Referring now to Figures 51A and 51B, a nozzle array 5100 of the present invention is shown. Therein, one or more spacer layers 5102 may be positioned between a desired number of to-be-formed channels, e.g., during stacking of the well structures.

[212] Referring to Figure 52, an enlarged cross section of stacked layers used to form the probes such as nano-probes having wells and tip portions with tip active area dimensions equal or less than the sub-objects being analyzed by the specimen, or of a nanometer or sub-nanometer scale for other applications as described herein. These tip portions are also formed to desired tip length, is shown. As described above, the layers 3838 have been processed to form the wells 3830 and nozzle tip regions generally by deposition of a layer 3838 of material capable of being selectively removed (e.g., etched) therein (the well) and thereon (the shelf at the top of the well), as described herein. The materials capable of being selectively removed for the plateau and and/or the well may be the same or different. The wells and plateaus have various dimensions that will characterize the nozzle array ultimately formed. The nozzle has a tip length NL, a tip opening height NO, and a period P.

[213] Note that the dimensions of such nozzles may be on the order of a less than a nanometer (e.g., less than 0.1 nm) to 10 or 10s of nanometers, on the order of 10 or 10s of nanometers to 100 or 100s of nanometers, or on the order of a tenth of a

microns or tenths of a micron to a micron or a few microns, depending on the desired application. Further, the arrays may be spaced apart by a few nanometers to several micros apart.

[214] Referring to Figure 53, an enlarged cross section of stacked layers used to form the micro and nano nozzles herein is shown, detailing grind stops 5386 provided to enhance the ability to control the nozzle length NL. In certain embodiments, it is desirable to minimize the nozzle length. A grind stop 5386 is provided proximate the desired nozzle length. The grind stop may be provided during processing of the wells on the device layer. Further, the grind stops may further serve as alignment marks, e.g., as described in aforementioned U.S. Patent Application Serial No. 10/717,220, incorporated by reference herein.

[215] Referring to Figures 54A and 54B, an enlarged cross section of stacked layers used to form the micro and nano probes, and a front view of the open tip of the prove, respectively, are shown. Note that in certain embodiments, the well 5470 has a width (y direction) greater than that of the nozzle tip 5468.

[216] Note that in any of the herein described probe elements, associated structures may be provided. For example, in certain embodiments, one or more electrodes may be provided to facilitate material discharge, detection capabilities, etc. Further, one or more processors, micro or nano fluidic devices, micro or nano electromechanical devices, or any combination including the foregoing devices may be incorporated in a nozzle device. In certain preferred embodiments, electrodes are provided at the nozzle openings and/or wells, and an electrode controller and/or a

microfluidic device (e.g., to feed or remove material from the nozzles) is associated with an array of nozzles.

[217] Further, and referring now to Figures 55A-D, an exemplary method of making probes with open tips and having various conductors (e.g., serving as electrodes) within an open region in the body fo the probe is depicted. Figure 55A shows a starting section of a multiple layer substrate with layers 5510 and 5520 as described hereinabove. An well 5530 generally has angular walls 5532 and a center recessed portion 5534, although other shapes may be provided. Plateau regions 5536 form the opening walls or supports.

[218] A layer 5538 of conductive material is deposited on the wafer. A removable fill material 5540 may be provided in the well to facilitate layering. Referring to Figure 55B, a removable fill layer 5542 is provided on the surface having the conductive layer 5538 and the optionally fill material 5540. In this embodiment, the opening of the probe will be formed at the fill layer 5542. Further, a conductive layer 5544 is deposited or layered on the fill layer 5542, forming a nozzle sub-structure 5550.

[219] Referring now to Figure 55C, a plurality of nozzle sub-structures 5550 are aligned and stacked (e.g., as described above with respect to Figure 42). Referring to Figure 55D, nozzle openings 5560 may be formed, e.g., according to one of the methods described above with respect to Figures 44-50, or other lithography or oxidation methods. Note that the plug material may be conductive or insulating, depending on the desired properties of the probe.

[220] Referring now to Figure 56, an enlarged view of a nozzle structure 5600 is provided, viewing a nozzle opening 5602. Nozzle opening 5602 is generally

positioned on a nozzle layer "N" between a top portion "A" and a bottom portion "B" (although top and bottom are considered to be relevant for the purpose of description herein only). To describe various embodiments of possible configurations, sections N, A and B have been divided into descriptive sections. These descriptive sections may be actual discrete regions of different material, or in certain embodiments multiple descriptive sections may be of the same material and thus actually a uniform region, as will be apparent from the various embodiments herein.

[221] AA and BB may be the same or different materials, such as insulator or semiconductor materials to provide the structure of the nozzle 200, electrically insulate the nozzle openings from one another, fluidly seal the openings from one another, or other functionality.

[222] In certain embodiments, the descriptive sections AL, AC, AR, NL, NR, BL, BC and BR are all of the same materials as AA and BB.

[223] Any combination of AL, AC, AR, NL, NR, BL, BC and/or BR may be provided in the form of conductors. For example, referring back to Figure 46, upon removal of the mask after etching the nozzle opening, a structure may be provided having AL, AC, AR, BL, BC and BR of the same materials as AA and BB, and NL, NR of conductive material.

[224] Further, one or more conductors (e.g., electrodes) may be included inside within the probes, thereby enabling creation of fields across the nozzle opening. For example, NL and NR, AC and BC, AL and BR, AR and BL, AL, AR and BL, BR may all be electrode pairs to provide any desired functionality. Additionally, one or more

conductive electrodes may be within the well regions, e.g., to provide electromotive forces to move materials.

[225] Referring now to Figures 57A-C, an example of a method of manufacturing the herein described nozzles is shown whereby a plurality of sub-layers 5702 form each layer 5710. Wells 5730 are processed through the layer 5710 as shown in Figure 57B. Figure 57C shows nozzle openings 5760 having plural sub-layers 5702 therearound. These sub-layers may be very useful, for example, where precise metrology is desired.

[226] For example, in certain embodiments, the sub-layers 5702 are formed to very precise tolerances, e.g., having thicknesses on the order of 0.1 to about 5 nanometers. When these sub-layers 5702 are formed of differing materials (e.g., alternating between insulator and semiconductor, semiconductor and conductor, or conductor and insulator), precise step motion may be enabled in the nozzle structures based on known dimensions of the nozzle sub-layers.

[227] While is is possible to use conventional lithographic tools such as electron beams, particle beams, UV, X-ay, etc., to define certain features herein, extending them to the nano-scale becomes very cumbersome and expensive. In the present invention ,certain embodiments may benefit from the use of applicants nanolithography tools described in applicants U.S. Patent Application Serial No. 11/077,542 filed on March 10, 2005 and entitled "Nanolithography and Microlithography Devices and Method of Manufacturing Such Devices" incorporated by reference herein. This is advantageous in that a company, easy to use and inexpensive tool may be proved.. Further, use of applicants nanolithography tools described in above referenced U.S.

Patent Application Serial No. 11/077,542 may advantageously provide extremely small future sizes down to angstrom scale.

[228] Various probes and configurations thereof may be manufactured with the use of Applicant's microlithography and nanolithography tools and methods, as described in U.S. Non-provisional Application Serial No. 11/077,542 filed on March 10, 2005 entitled "Nanolithography and Microlithography Devices and Method of Manufacturing Such Devices".

[229] In certain embodiments herein, a probe may be formed by folding a very thin layer to expose a point at the outside of the fold angle, thereby creating a probe tip with a very small active area suitable for the various applications provided for herein including ultra high resolution analyses of the specimen at the sub-object level (e.g., nucleotide level of a DNA or RNA strand or fragment).

[230] For example, and referring now to Figures 58-60, a method of manufacturing a probe 5802 is shown. Figure 58A shows an ultra thin layer 5804 bonded to a first surface 5808 of a base layer 5806. The base layer 5806 may comprise any suitable material, for example, that will form a portion of the probe body, or that may be further processed for additional features and/or functionality. The ultra-thin layer 5804 may comprise any suitable material that may be deposited, laminated or otherwise formed on the surface 5808 of base layer 5806.

[231] Referring now to Figure 58B, a well 5812 of suitable geometry is etched or otherwise created on surface 5810 of base layer 5806. In certain embodiments, it may be desirable to configure the well such that the deepest portion is very close to the thin layer 5804. In other embodiments, it may be desirable to configure the well such

that the deepest portion exposes the back surface (i.e., the surface attached to surface 5808 of base layer 5806) of the thin layer 5804.

[232] Referring now to Figure 58C, surface 5810 may optionally be coated with a bending layer 5814 formed of a material that has flexible characteristics, including but not limited to polyvinyl alcohol, silicone, or other suitable flexible and stretchable polymeric or other materials.

[233] Referring now to Figure 58D, the composite of layer 5804 and base layer 5806 is folded to diverge opposing angled portions of the well 5812. Folding is completed to provide a probe precursor structure 5802', shown in Figures 59A and 59B. As shown, the probe precursor structure 5802' has a cross section in a substantially pentagonal shape resembling a triangle adjacent a rectilinear polygon. Of course, one may alter this shape by changing the shape of the well 5812. Further, the shape may be symmetrical as shown, or asymmetrical. One of the benefits of the present folding techniques is that certain alignment requirements may be relaxed.

[234] The bending layer 5814 may be removed. Further, to expose the probe tip active area 5820, the tip edge 5816 of the structure 5802' may be grinded, polished, or otherwise removed to expose the folded thin layer of material. The dimension of the probe tip active area 5820 is defined by a multiple of the thickness of the layer 5804, in this case $2t$.

[235] Notably, with the methods of making and manipulating thin films as described above, extremely small tip dimensions for the probe tip active area are possible. For example, if the layer 5804 is a single two dimensional layer of graphene,

then the tip dimension $2t$ as shown in Figure 60 may be on the order of 2 angstroms, and is highly conductive.

[236] Alternatively, the layer 5804 may be formed of a material that can be selectively removed (either completely or partially) to open a channel or path. Nonetheless, in either embodiment, the tip dimensions for the tip active area 5820 are a multiple of the thickness of the layer 5804 deposited, layered, or otherwise formed on the base layer 5806.

[237] In another embodiment, and referring now to Figures 61A-61J, methods of manufacturing a probe 6102, 6102' or 6102'' are shown. Figure 61A and 61B show a base layer 6106 having a well 6112 of suitable geometry is etched or otherwise created on surface 6110 of base layer 6106. In certain embodiments, it may be desirable to configure the well such that the deepest portion is very close to the thin layer described below. In other embodiments, it may be desirable to configure the well such that the deepest portion exposes the back surface (i.e., the surface attached to surface 6108 of base layer 6106) of the thin layer described below.

[238] The base layer 6106 may comprise any suitable material, for example, that will form a portion of the probe body, or that may be further processed for additional features and/or functionality.

[239] Referring now to Figure 61C, portions 6124 are removed from the base layer 6106, generally from the side of surface 6108. Referring now to Figure 61D, portions 6124 are filled with a suitable material 6126. This material 6126 may be an insulating or conducting plug material (if a probe in the configuration of Figure 61H or

61I is desired), or the material 6126 may comprise a removable substance (if a probe in the configuration of Figure 61J is desired).

[240] Referring now to Figure 61E, an ultra thin layer 6104 bonded to the surface 6108 of base layer 6106 having material portions 6126 to form a flat surface. Known techniques may be applied to smooth the surface formed by both the surface 6108 of base layer 6106 having material portions 6126. Alternatively, the methods for forming atomically smooth surfaces described herein may be employed.

[241] The ultra-thin layer 5804 may comprise any suitable material that may be deposited, laminated or otherwise formed on the surface 5808 of base layer 5806. In certain preferred embodiments, thin films formed according to the embodiments herein are used.

[242] Notably, with the methods of making and manipulating thin films as described above, extremely small tip dimensions for the probe tip active area are possible. For example, if the layer 6104 is a single two dimensional layer of graphene, then the tip dimension is $2t$ as shown above in Figure 60.

[243] Alternatively, the layer 6104 may be formed of a material that can be selectively removed (either completely or partially) to open a channel or path. Nonetheless, in either embodiment, the tip dimensions for the tip active area 6120 are a function of the thickness of the layer 6104 deposited, layered, or otherwise formed on the base layer 6106.

[244] Referring now to Figure 61F, surface 6110 may optionally be coated with a bending layer 6114 formed of a material that has flexible characteristics, including

but not limited to polyvinyl alcohol or other suitable polymeric or flexible metallic material.

[245] The composite of layer 6104 and base layer 6106 having material portions 6126 is folded to diverge opposing angled portions of the well as described above with respect to Figure 58. A probe or probe precursor structure 6102 is provided as shown in Figure 61H (after the material of the optional bending layer 6114 is removed as shown in Figure 61G). If a probe 6102' is desired, the selectively removable material is used as the material for layer 6104, and may be removed at this stage, whereby a gap 6128. Further, if probe 6102'' is desired, the selectively removable material is used as material 6126 and may be removed at this stage, whereby a cavity 6140 is created.

[246] Referring to Figures 62A-62B, note that cavities of various configurations and dimensions 6240', 6240'' may readily be created by varying the configurations and dimensions of portions 6124 described above with reference to Figures 61A-61J.

[247] Referring now to Figure 63A-63D, another alternative method of making various probes with additional versatility and functionality according to the present invention is provided. In this case, the structure having a thin layer 6304 on a base layer 6306 with a well 6312 at the surface opposite the thin layer 6304 is folded so that angled portions of the well 6312 converge as shown. The bending layer or material 6314 may be removed, resulting in probe 6302 having a tip 6340.

[248] Referring now to Figure 64, a probe 6410 as formed by various aspects of this invention may be utilized to assist in the folding. For example, probe 6410 may be used to contact within the well, whereby the mechanical forces assist in the

folding processes. In further embodiments, vacuum suction may be applied through the probe 6410 to assist in the folding processes.

[249] Referring now to Figure 65, a plurality of probes 5802 may be aligned and stacked, for example, by stacking edgewise on a platform 6530 and aligning by stacking the tips of the probes 5802 adjacent an alignment device 6534, or stacking the probes 5802 and displacing misaligned probes 5802 by pushing them into alignment with the alignment device 6534, thereby forming probe sets or probe arrays. In certain preferred embodiment, alignment device 6534 has a surface that contacts the tips and provides for sub-angstrom resolution motion to precisely displace and align the tips of the probes in an array or probe set. It is particularly advantageous if the surface that contacts the tips is atomically flat and smooth, for example, as may be produced by various methods described herein.

[250] Referring now to Figures 66A-66D and Figures 67A-67E, another method of forming probes according to the present invention is shown, particularly open tip probes. In particular, the tip opening dimensions t are defined by use of spacers such as particles, tubes, spheres, molecules, or other structures having precisely defined heights when disposed on a substrate. These spacers may have extremely small defined dimensions (e.g., a diameter of a sphere or tube that provides the height), such as in the ranges of 0.1 nanometers to about 10 nanometers, 10 nanometers to 100 nanometers, and 100 nanometers to 1000 nanometers.

[251] In one example, and referring to Figure 66A-66D, a plurality of spacers 6614 are disposed generally in an orderly fashion upon the surface of a substrate 6610. As shown in Figure 66A, the spacers 6614 may, for example, be aligned in groups

along the x direction and spaced apart from one another in the y direction. Alternatively, the spacers 6614 may be in a contiguous form.

[252] Referring now to Figure 66B, a superstrate 6620 is provided on the spacers 6614 to complete the probe or probe precursor by defining an opening 6624. Alternatively, and referring to Figures 66C and 66D, the probe may be cut into segments as shown by dashed lines in Figure 66C.

[253] In another example, and referring to Figures 67A-67B, a plurality of spacers 6714 are disposed generally in a random fashion upon the surface of a substrate 6710. Referring now to Figure 67B, a superstrate 6720 is provided on the spacers 6714 to complete the probe or probe precursor by defining an opening 6724.

[254] Referring now to Figure 1, a schematic overview of a system of the present invention for analyzing extended object specimens is shown. The system 100 generally includes a specimen platform 128, a probe set 130 and a detector sub-system 132. The platform 128 is operably coupled to a motion controller 138, for controlling motion of the platform. Alternatively, the specimen may be moved within the platform. In a further alternative, the probe set (and optionally the associated detector sub-system) may be moved relative to the platform with the specimen. Further, the system 100 includes a bias sub-system 136 for control of field application (voltage applied across base and probe) and optionally other stimuli. In general, in certain systems described herein, when a hybridization event occurs, a measurable increase in current is detected.

[255] In certain embodiments, a low detection voltage may be applied in a constant manner across the probe set and the platform. However, biased voltage application may be utilized to minimize or eliminate noise.

[256] Data regarding the specimens is collected and processed by a processor sub-system 134, which is coupled to an output sub-system (e.g., a display, data port, etc.) 140.

[257] In operation, a specimen such as a single stranded polymer (e.g., a denatured strand of DNA) is directed through a path or channel in the platform. The probe set detects characteristic features of the polymer specimen, preferably detecting characteristic about each sequential monomer in the specimen polymer. The specimen is moved relative the probe set in a controlled manner, e.g., by step motion to allow the probe set to obtain characteristic information about each monomer or group of monomers. The sequence information is collected, processed and outputted.

[258] In certain embodiments, high resolution is attainted by utilizing a probe having a tip dimension, or an active tip area, that is equal to or less than a characteristic sub-object of the extended object, such as a nucleic acid within a DNA or RNA strand or fragment. In further embodiments, the width dimension of the probe is much larger than the width of thickness of the extended object, for example, having width *w* of about 10 nanometers to about 100 nanometers, 100 nanometers to 1000 nanometers, or several microns for analyzing specimens such as typical DNA strands or fragments. Further, the enlarged width dimension as compared to the tip or active area is useful in that additional tolerance is provided for the path of channel of the specimen and/or the stretching procedures.

[259] Referring now to Figure 68, an embodiment of an ultra-fast DNA sequencing system 6800 is shown. The sequencing system uses a nozzle array 6810, as

described herein. Further, the sequencing system uses a nano-metrology system 6820 to precisely guide denatured DNA strands across the individual nozzles in the nozzle array.

[260] Referring now to Figure 69, a schematic of major components of the ultra-fast DNA sequencing system 6800 are shown. A nano-nozzle set array platform 6830 upon an N-channel specimen array platform 6828 is operably connected to a detector array 6832 associated with a processor 6834, generally for determining instances of hybridization events induced by the biases applied via a gated bias array control 6836. The DNA specimens are maintained and displaced in relation to the array with a stepped motion control 6838, which is also operably connected to the processor 6834. The array platform 6828 is movable at a velocity of about 0.1 to about 1 cm/s. Preferably, as shown, the motion is in a stepped manner, as described herein. The sequencing results are shown on a sequence display 6840.

[261] The stepped motion is important in preferred embodiments, as the motion and number of steps helps maintain knowledge of position on the ssDNA, and ultimately the position of hybridization events. The stepped motion may be from about 5% to about 100% of the nozzle opening dimension, preferably about 10% to about 25% of the nozzle opening dimension.

[262] The gating is also important in preferred embodiments, as extremely synchronized current measurements, bias, motion steps, or other excitations are crucial to ultra-fast real time DNA sequencing.

[263] Referring now to Figure 70, a top view of the ultra-fast DNA sequencing system 6800 is shown. The DNA specimens are denatured and maintained within channels 6844.

[264] Referring now to Figures 71A-B (wherein Figure 22A is a section along line A-A of Figure 70), each channel 6844 includes biasing systems for applying voltages across the DNA samples. As described in more detail herein, hybridization events induce measurable current variations across each of the nanonozzles within the nanonozzle set array platform. Preferably, the alignment between the nanonozzles and the channels is extremely precise.

[265] Referring now to Figures 72A-C, a system 7200 including series of probe sets 7230, a probe set 7230 including nozzles or probes 7242, 7244, 7246 and 7248, and an enlarged view of probe 7248 are shown. The nanonozzle set array platform 7200 includes nanonozzles with wells, or nucleotide reservoirs, of A, C, T and G molecules. The strands are moved along the channel and molecules from the nucleotide reservoirs interact with the molecules of the strand through the nozzle. These molecules hybridize with one other molecule (e.g., A with T, C with G). In general, the hybridization event (e.g., as shown in Figure 72C) produces measurable and detectable current pulses, thereby allowing identification of the molecule.

[266] Referring now to Figure 73, detailed views of hybridization events are shown. In certain detection schemes described herein, a hybridization event at the nanonozzle results in a measurable current pulse.

[267] Referring now to Figure 74, it is shown that, of all possible 16 combinations of A,T,G and C, only four produce desired current pulses upon a hybridization event.

[268] As mentioned above, only a hybridization event produces a measurable (nanoseconds) current pulse at the nozzle. For optimized operation, the following principles apply.

•All excitation sources, detectors and stepped motion are synchronized.

•Synchronized steps should be a fraction of the nozzle opening size (e.g., on the order of 5 nanometers).

•Nozzle locations should be known with nanometer or sub-nanometer precision in relation to a known reference position.

•Nanometer alignment is very important to optimal operation.

•Vibrations and other agitations should be minimized.

•A sub-system is provided to measure very low amplitude nanosecond pulses.

•For continuous real time measurement of millions, or even hundreds of millions, of base pairs, a wide dynamic range sub-nanometer stepper is preferred.

•To calibrate the system, it is desirable to use known samples.

[269] In a preferred embodiment, the probes in the form of electrode conductors and/or other stimuli are applied in a gated manner. This reduces the signal to noise ratio thereby allowing for increased sensitivity and ability to resolve the sequence of the specimen.

[270] Detection of a hybridization event may be accomplished in certain embodiments by observing variations in resonant capacitance. For example, an AC bias is imposed through a probe and a grounded platform (or alternatively AC bias may be imposed through the platform and the probes are sequentially grounded). The AC bias will alternately deplete and accumulate the specimen. The change in capacitance ΔC is

recorded, for example, using a lock-in technique. The measured value ΔC may be the value across the entire C-V curve when larger AC voltages are used, or measured value ΔC may be the differential capacitance dC/dV when smaller AC bias voltage is used. The variation in the load across the specimen occurs due to characteristics of the portion of the specimen to be resolved such as a monomer on a polymer strand, or due to creation of a hybridization event when the probe includes a hybrid pair counterpart. This load variation changes the resonant frequency of the system.

[271] Electrical conductors as probes according to preferred embodiments of the present invention, formed as described above with respect to Figures 2 and 3 above (e.g., in the configuration with a very fine tip compared to the back end, or a "knife edge") also serves to lower the resistance of the conductor.

[272] Various embodiments of stimuli application are possible. 1) voltage only; 2) voltage plus light (AND gate) (light is a noise reduction means); 3) synchronization with gating, pulsed voltage, light, and current gate leads to substantial noise reduction; 3a) controlled stepping; 3b) apply voltage and light (AND gate) – light of different wavelengths to enhance inelastic tunneling current; 3c) apply current gate (measure with ammeter); 4) kT (thermal energy) may be reduced under low temperature operating conditions, e.g., T between 4 and 100 K.

[273] Gated detection serves to minimize noise and allow for precise resolution of the extended object. Gated detection is necessary to ensure the detection of picoamp level currents in the presence of noise. One effective strategy is to apply all of the stimuli in the proper sequence, in the form of pulses. The pulse widths and heights are adjusted to achieve optimum results. The levels of voltage will be in the 10s of

millivolts up to about 1 volt. The pulse durations may be about 1 nanosecond to about 1000 nanoseconds, or longer if necessary.

[274] The protocol for gated detection is described in the following steps: 1) apply a pulse to step the specimen relative to the platform to a position to measure a portion of or a nucleotide of the specimen; 2) subsequent application of an electric field to provide contact between the specimen and the probe; 3) optional application of a laser pulse; 4) application of tunneling device voltage pulse; 5) applying a pulse to open the switch to the current measure device; 6) repeating 1-5 to measure the subsequent portion of the specimen or nucleotide to sequence. These steps 1-5 are synchronized pulses synchronized to a master clock. In the event that particle beams are applied, or intensifiers, these will also have appropriately applied excitation pulses to activate them synchronized with said clock. These gated synchronized methods allow one to measure the detectable interaction with a high signal to noise ratio.

[275] For example, referring now to Figure 77, a sampling period 7700 of a series of synchronous excitations are charted on a plot 7702 relative to clock signals 7710. A stepping period is shown as a short pulse commencing at a certain time indicated on a horizontal axis 7720, e.g., at the start of the sequence. A contact period is shown as commencing after the stepping period as indicated on a horizontal axis 7730 and ending during of after measurement and/or processing and storage periods. A photon period is shown as increasing in amplitude after the commencement of the contact period as indicated on a horizontal axis 7740 and ending proximate the end of the contact period. A voltage bias period is shown as commencing during the photon period as indicated on a horizontal axis 7750 and ending proximate the end of the contact period. A current

detection period is shown as commencing during the photon period and the voltage bias period as indicated on a horizontal axis 7760 and ending proximate the end of the contact period. A processing and storage period is shown as commencing near the end of the photon, voltage bias and current detection periods as indicated on a horizontal axis 7770 and ending after the end of the contact period.

[276] Detection of the portion of the specimen under examination may occur by various contribution. In general, the detection schemes allow for molecular level (or detection of one or more monomers, or certain groups of monomers, in an extended object to be analyzed) identification of monomers within a chain.

[277] In a single strand specimen analysis systems having probes that induce a hybridization event, detection contribution includes elastic tunneling, inelastic tunneling, resonantly enhanced tunneling, and/or capacitance. Figure 80 shows the typical Watson and Crick base pairing model. Referring now to Figure 81, a system 8105 schematically shown including a probe 8110 and a substrate 20 having a specimen 8130 thereon. The probe is designed to induce a hybridization event, as described herein, by including a complementary specimen in a well, on a substrate, or by other configurations. A voltage bias is applied, for example, that corresponds to the N-H bonds and O-H bonds formed during a hybridization event.

[278] The elastic tunneling contribution in systems having probes that induce a hybridization event is generally due to the tunneling interaction variations that occur due to the distance between hybridized species. When a hybridization event occurs, the distance between the hybridized monomers (nucleotides) is modulated as the bond is created. As the tunneling barrier thickness decreases, tunneling probability

increases and thereby increases the tunneling contribution. This will be manifested in the increase of conductance as measured in the current-voltage characteristics of the hybrid bond. When no hybridization event occurs, the distance between the probe capable of inducing a hybridization event and the specimen nucleotide remains relatively large, and hence the elastic tunneling contribution is relatively low.

[279] Referring now to Figure 82, system 8205 schematically shown to illustrate the elastic tunneling contribution. When a bond is established as a result of the relatively shorter distance (thinner tunneling barrier) that results for the H-Bond. This manifests itself as an increase of the conductance, and hence higher current. Note this elastic tunneling contribution generally does not involve exciting a resonance.

[280] The inelastic tunneling contribution in systems having probes that induce a hybridization event is based on increased bond energies, especially hydrogen bond energies. During a hybridization event, as electrons tunnel, the electrons lose energy by exciting the hydrogen bond created as a result of the hybridization event. This leads to a tunneling contribution at a voltage correlating to the energy of the bond. When no hybridization event occurs, there is no hydrogen bond created, therefore there is no inelastic tunneling to excite such a bond, and therefore no conductance contribution should be observed.

[281] Referring now to Figure 83, system __05 schematically shown to illustrate the inelastic tunneling contribution. In addition to the above increase in current due to the elastic tunneling contribution, another increase will be detected due to an inelastic tunneling contribution resulting from exciting the resonance of the H-Bond.

[282] The above may enhanced by applying a source tuned to the bond frequency, thus providing an optically enhanced inelastic tunneling contribution. For example, as described above with regard to Figure 28, a tune light source may be applied in conjunction with the measurement bias. This optically enhanced inelastic tunneling component contributes to minimizing the noise effect by acting as and "AND" gate, such that current signal detection is primarily when synchronous application of the optical signal "AND" the bias voltage (both tuned to the resonance).

[283] Referring now to Figure 79, another embodiment of the present invention is shown. A specimen portion 7910 is within a probe system 7920 including a first probe 7930 and a light nozzle 7950. The light nozzle 7950 and the first probe 7930 are activated, either sequentially, simultaneously or overlapping in time, to facilitate current detection, measurement, or other impact of the detection contribution effect, as described above. The first prove 7930 may include any one of the above referenced types of probes. Alternatively, more than one probe may be used with a cooling droplet supply nozzle 2740, for example, for photonic application, current measurement, voltage bias, or other functionality as described herein.

[284] The resonantly enhanced tunneling contribution in systems having probes that induce a hybridization event is based on measurement of excited bond energies, particularly hydrogen bonds. Stimuli such as light application is applied. A resonantly enhanced tunneling contribution may be observed when a light source such as a laser having a suitably tuned wavelength excites the hydrogen bond created upon hybridization. Hydrogen bonds from the hybridization events can be excited by tuning a laser beam to the same energy as the bond. This will enhance the detection of both the

elastic and inelastic tunneling contribution and add a resonant enhanced tunneling contribution to the measurement current. Further, noise is minimized with suitable gating as described herein since the pulsed application of the laser light source is synchronized with application of a voltage and during the opening of the measurement current sensor. These simultaneous interactions have the effect of a logical "AND" gate.

[285] The capacitance contribution in systems having probes that induce a hybridization event is based on enhanced permittivity. Since the tunneling area is very small, the application of a laser beam tuned at or near the bond energy creates a resonantly enhanced permittivity at the hybridized pair. This in effect is like a quantum capacitance. This quantum capacitance, added to a specific inductive element, an RF resonant circuit, or a RF resonant cavity, results when the hybridization even occurs. For example, the inductive element, RF resonant circuit or RF cavity are excited and can give a very large signal. Since RF frequencies are at higher frequencies than the DC voltages, there is low noise in that region (avoiding the 1/F noise).

[286] Referring now to Figure 84. system __06 schematically shown to illustrate the quantum capacitance contribution. The quantum capacitance contribution is a result of enhancement of polarizability of molecules by exciting suitable resonances, including O-H or N-H bonds, and further rotational, vibration, and electronic. These are represented in Figure __ by resonances $\hbar\omega 1$, $\hbar\omega 2$, and $\hbar\omega 3$. The energy is represented by:

[287] $Eqc = \frac{1}{2} (Cq\ V2)$.

[288] RF measurement is conducted using special resonance circuits that include "quantum capacitance" which will be enhanced when O-H or N-H resonances are

excited by external radiation tuned to these resonances. This is expected because the capacitance is related to the permittivity of the interaction between the probe __10 and the sample __30. This permittivity has a susceptibility component which in turn is given by the polarizability at the molecular level. The value of this polarizability has many resonant contributions, including vibrational, rotational, and electronic. It is well known that if any one of these resonances - vibrational, rotational, or electronic – are excited, even away from the specific bonds, a significant increase in the polarizability, and hence the capacitance, results. The optimum tank circuit, e.g., in microwave or millimeter wave, will be excited and detected. Since these are high frequencies, we will be far away from the 1/f noise regime, thus the signal to noise ratio is large.

[289] In a single strand specimen analysis systems having probes that do not induce a hybridization event, detection contribution includes inelastic tunneling, resonantly enhanced tunneling, and/or capacitance.

[290] Detection based on the elastic tunneling contribution is not particularly effective without a probe that induces a hybridization event. Since the distance between the probe (in a system that does not induce a hybridization event) and the specimen nucleotide reaming relatively large, the elastic tunneling contribution is relatively low for all nucleotides. Therefore, an elastic tunneling contribution is not suitable for measurement detection system when using probes that do not induce hybridization events.

[291] However, detection of measurement current variances due on inelastic tunneling contribution may be used. Since there is no hybridization event (e.g.,

the probes are formed of conductors or other style that does not induce a hybridization event), we rely on the inherent resonance of each nucleotide to be analyzed.

[292] Further, the resonantly enhanced tunneling contribution is suitable, wherein a light source (e.g., laser wavelength) is tuned to the inherent unique resonances of the nucleotides to be analyzed. The nucleotides to be analyzed are be excited by tuning a laser beam to that unique resonance, which will enhance the detection of the inelastic tunneling contribution and other contributions to the current measurement. Further, noise is minimized with suitable gating as described herein since the pulsed application of the laser light source is synchronized with application of a voltage and during the opening of the measurement current sensor. These simultaneous interactions have the effect of a logical "AND" gate.

[293] The capacitance contribution in systems having probes that do not induce a hybridization event is also based on enhanced permittivity analysis. Since the tunneling area is very small, the application of a laser beam tuned at or near the inherent unique resonance energies creates a resonantly enhanced permittivity of the signature. This in effect is like a quantum capacitance. This quantum capacitance, added to a specific inductive element, an RF resonant circuit, or a RF resonant cavity, results when the signature energy occurs. For example, the inductive element, RF resonant circuit or RF cavity are excited and can give a very large signal. Since RF frequencies are at higher frequencies than the DC voltages, there is low noise in that region (avoiding the 1/F noise).

[294] In other embodiments of the present invention, instead of, or in conjunction with, measuring a current variation,

[295] Use probe , bring close to specimen, at known distance, attraction force will be detected. Rather than detect current flowing there through, detecting attractive or repulsive motion.

[296] Knife edge AFM probe – contacts specimen, measures attractive or repulsive forces

[297] It is well known that atomic force microscopy (AFM) is used to analyze nano-structures an atomic scale. One key element leading to the success of the AFM is attachment of a nano-tip to a cantilever that is made to deflect when the nano-tip measures forces of the interaction between said nano-tip an the structure under analysis. A laser beam reflecting from the cantilever measures the forces variations as the nano-tip scans the structure.

[298] By utilizing the inventive embodiments taught herein, it is possible to analyze an extended object such as a DNA sequence by measuring the force as in AFM , instead of or in conjunction with the tunneling currents. This is shown in Figure {AFM1}. Here the attractive force that results when A bonds with T and C bonds with G as a result of hybridization is relied upon to detect certain species. The specificity of the sequencing is accomplished by utilizing a probe with characteristics that allow it to attract certain species, such as by attaching poly-A, poly-T, poly-C, and poly-G oligomers to nano-edge probes, for example, as described herein. Each of the 4 nano-edge probes is attached to a different cantilever. A detector measures the deflection of each different cantilever which modulates the reflection of laser beams of a different wavelengths in the response to the interactive forces between the edge or tip nano-probe and the specimen to be analyzed.

[299] The AFM sequencing processes and systems described herein may be further described by the following. An extended object such as a single strand DNA (SSDNA) is stretched and immobilized on a substrate. A sub-Angstrom resolution translation stage moves the specimen relative to the set of edge-nano-probes.

[300] The edge nano-probe with the poly-A attached to it will experience and attractive force when it is proximate to or lands on the specimen with a T base. This force will modulate the reflection of the laser beam of wavelength λ_A by the cantilever. The modulated reflected beam announces the presence of T at that location with the aid of a detector and processing electronics.

[301] The edge nano-probe with the poly-T attached to it will experience and attractive force when it lands on the specimen with a Abase. This force will modulate the reflection of the laser beam of wavelength λ_T by the cantilever. The modulated reflected beam announces the presence of A at that location with the aid of a detector and processing electronics.

[302] The edge nano-probe with the poly-C attached to it will experience and attractive force when it lands on the specimen with a G base. This force will modulate the reflection of the laser beam of wavelength λ_C by the cantilever. The modulated reflected beam announces the presence of G at that location with the aid of a detector and processing electronics.

[303] The edge nano-probe with the poly-G attached to it will experience and attractive force when it lands on the specimen with a C base. This force will modulate the reflection of the laser beam of wavelength λ_G by the cantilever. The

modulated reflected beam announces the presence of C at that location with the aid of a detector and processing electronics.

[304] The edge nano-probes with the poly-A , poly-T, poly-C, or poly-G will experience a weaker (no force or repulsive) force when either non complementary base, e.g. A on A, T on T, C on C, G, on G, A on C, A on G, T on C, or T on G. In these cases the beams reflected from the cantilevers will have small force modulation.

[305] It is possible to use a single laser beam that is divided into 4 beam-lets, each is focused on different cantilever at certain positions, to minimize interference. This detector will specially resolve the positions of the beam-lets so as to differentiate and ensure specificity.

[306] Auxiliary laser beams may optionally be focused on the specimens, for example, that are tuned to certain frequencies that interact with the specimen. This can enhance the specificity and reduce errors of ambiguity.

[307] Instead of using 4 nano-probes in parallel whereby each reflects its own laser beam or beam-let, it is possible to have nano-probes that are inserted or activated sequentially. For example, an embodiment is this system is illustrated in Figure {AFM2}. Here the probes are attached to a rotating mechanism (e.g., "daisy wheel style") which rotates to expose the probe to the specimen one at a time. To sequence a DNA specimen, the probe functionalized with the poly-A oligomers is inserted (rotated in) and will scan the specimen. Then the poly-T is inserted to record the positions of the A nucleotide. This is repeated for the C and G nucleotides until the entire specimen is scanned with the four probes and the sequencing is completed. As shown in Figure {AFM3}, this apparatus may be made more general and versatile by attaching to the

daisy wheel a plurality of probes with different shapes, knife edge, single point, multiple tips, different functional group to recognize specific species, and nano-crystals of specific composition designed to search for and locate a specify material. This versatility is particularly useful as it affords the opportunity to use the system as an imaging tools first, as in normal AFM, then as a sequencing tool or more generally a chemical analysis tool.

[308] It is appreciated that instead of a daisy wheel arrangement, there may other more advantageous arrangements. In order for these apparatuses with sequential insertion of probes to function properly, precise alignment subsystem may be required located with precision a spatial reference point, relative to which all spatial information is recorded. This will minimize errors and ambiguity. Additional nano-probes may be attached to function as the locators of alignment marks purposely written on the substrate.

[309] As descried herein, array of probes sets in 2d or 3d arrays can measure and re-measure the same sample., This is possible due to the low cost techniques. Further, multiple channels for parallel systems may be used.

[310] As descried herein, array of probes sets in 2d or 3d arrays can measure and re-measure the same sample., This is possible due to the low cost techniques. Further, multiple channels for parallel systems may be used.

[311] In another embodiment, and referring now to Figure {DD1}, a system is provided to use differential detection to minimize errors in reading the sequence. Arrays of nano probes/nozzles affords the opportunity, inexpensively, to consider repeated measurements to minimize the noise. For example, differential detection strategies may be used whereby system noise may be subtracted in real time.

One or more probes or probe sets read the specimen and known samples A,C,T,G. Accuracy may be increase by performing differential detection, whereby noise may be determined and subtracted from the specimen reading. For example, we may read synonyms with the specimen analysis a current of a known sample (e.g., Arrays of A, C, T, and G). This gives us noise and the contribution of T at a particular instant of time. At the same instant of time, if a T is apparently determined to be the base of the specimen, the noise may easily be subtracted to confirm that the reading of T is accurate.

[312] Therefore, the following apply:

- Current (known sample) = noise+ contribution of T (apply positive pulse)
- Current (specimen) = noise - contribution of T (apply negative pulse)

[313] The contriubiton of the signal is detected at certain modulation frequency, whereas the noise is random

[314] AAA, GGG, TTT, CCC also could be known AGAGAGAG, TCTCTCTC, so long as it is known.

[315] Many sensing techniques for determining a hybridization event include elastic quantum mechanical tunneling; inelastic quantum mechanical tunneling; resonantly enhanced tunneling; resonantly enhanced quantum capacitance in a tank circuit to boost the signal of hybridization events; fast cooling techniques to reduce noise (for example, such as the system that utilized liquid He or liquid N_2 droplet cooling); ionic conductivity; quantum mechanical tunneling electron emission; photon emission, which can be amplified by photon multiplier techniques. Any one or more of these techniques may be used in conjunction with the herein described high spatial resolution

(e.g., nucleotide monomer level resolution) probes, probe sets or probe arrays as a novel direct sequencing system.

[316] In certain embodiments, fast cooling techniques may be incorporated. As shown in Figure 78, for example, a specimen portion 7810 is within a probe system 7820 including a first probe 7830 and a cooling droplet supply nozzle 7840. The cooling droplet supply nozzle 7840 may include liquid He, liquid N2, or other suitable coolant suitable for fast cooling application. The first probe may include any one of the above referenced types of probes. Alternatively, more than one probe may be used with a cooling droplet supply nozzle 7840, for example, for photonic application, current measurement, voltage bias, or other functionality as described herein.

[317] Another aspect of the present invention to minimize error is the extended configuration (e.g., "knife edge") as described above with respect to Figures 2 and 3.

[318] In systems herein where metal contacts or probes are used to measure currents and voltages from small structures such as the monomers of the specimen, four probe tunneling devices may be used (e.g., shown in Figure 17) to minimize contact and lead resistance. Also, preferred probe configuration provide for a larger end opposite the tip, for example, as shown with respect to Figure 2. Further, all contacts the probe are preferably much larger than the tip. This can, for example, reduce electrical resistance of the probe when end serves as a contact region.

[319] Optimum specimen resolution and speed may be achieved by optimizing the detection system to increase the measurable signal, namely, ensuring that enough electrons are involved, and minimizing the ambient noise. The tunneling current

densities involved, in such small tunneling areas (e.g., .5 square nanometers), makes it possible to involve 10s of electrons and 10s of picoamps. This is achieved by allowing the time aperture to excite and detect each nucleotide in the order of 1 – 1000 nano-seconds. This can achieve the desired result of sequencing the whole Human Genome of 3×10^9 base pair in a time of about 1 second to a few minutes.

[320] We have allowed for even higher speed and fewer electrons to be involved whereby intensification/amplification sub-systems are used to intensify few electrons or photons into a measurable signal.

[321] Gated electronic techniques are also used herein with a pulse protocol that is applied to ensure minimize noise. This is desirable to ensure the detection of picoamp level currents in the presence of noise. One effective strategy is to apply all of the stimuli in the proper sequence, in the form of pulses. The pulse widths and heights are adjusted to achieve optimum results. The levels of voltage will be in the 10s of millivolts up to about 1 volt. The pulse durations may be about 1 nanosecond to about 1000 nanoseconds, or longer if necessary.

[322] The protocol for gated detection to minimize noise is described in the following steps: 1) apply a pulse to step the specimen relative to the platform to a position to measure a portion of or a nucleotide of the specimen; 2) subsequent application of an electric field to provide contact between the specimen and the probe; 3) optional application of a laser pulse; 4) application of tunneling device voltage pulse; 5) applying a pulse to open the switch to the current measure device; 6) repeating 1-5 to measure the subsequent portion of the specimen or nucleotide to sequence. These steps 1-5 are synchronized pulses synchronized to a master clock. In the event that particle

beams are applied, or intensifiers, these will also have appropriately applied excitation pulses to activate them synchronized with said clock. These gated synchronized methods allow one to measure the detectable interaction with a high signal to noise ratio.

[323] In another embodiment, referring now to Figure 8C, a plurality of nano-probe sets are provided, wherein each nano-probe set is specific to a certain species (e.g., nucleotide). The specimen is measure several times (by each probe within the probe set) and stored by a the first single species probe set. The specimen is then sequentially measured with a second single species probe set, a third single species probe set, and a fourth single species probe set to obtain data from each group of probe sets and obtaining at least one hybridization event or other detection event, preferably duplicate events to ensure accuracy of determination. Each probe set may and the computer analysis provides a consensus of the identity of the species, after averaging or other suitable statistical analysis. Each species is measured several times by one group, then another, then the 3rd, then the 4th. For example, if a probe set is optimized to detect an event with a T species, the following detection readout may be determined at that probe set for that base: TTCT. As the specimen and hence a particular base is moved across the array of 4 probe sets, A/T/C/G, the the following detection readout may be determined at that probe array for that base: TTCT/- -G -/C- - -/- - A -. Thus, some of the individual probes within the sets may provide erroneous results (e.g., the C within the first TTCT, the G within the second group, the C within the third group and the A within the fourth group), statistical analysis will determine that the particular base is indeed a T base. Note that more or less probes that four may be in each probe set. Further a scheme may be provided with various degrees of redundancy, including differing numbers of

probes within the probe sets, combinations of homogeneous and heterogeneous probe sets, combinations of probe type for various detectable Interactions (e.g., nucleotide filled wells, solid state nucleotides, metal conductor, metal plus known nucleotide stand, open well or funnel for particle beams, electron beam emission, ion beams, x-rays or the like, or flexible membrane probes.

[324] One important factor of these method and strategies for error reduction is obtaining a sufficient signal to noise ratio. The system is preferably gated and synchronized such that the ammeter will only detect a signal when a nucleotide is directly below a nozzle. The bias applied may be positive, negative, or even alternating, as to maximize the change in conductivity. Cooling may be desirable to reduce the thermal noise. Alternatively, each DNA or protein strand may be passed under several arrays of nozzles, thereby averaging out the noise. Certain embodiments show array configurations, e.g., that may average out noise and increase SNR. These features will help in assuring an excellent SNR.

[325] However, if we assume a 10 picoamp current change under one applied volt, and 10 nanoseconds for detection, the signal is orders of magnitude larger than the thermal noise, even at room temperature. The sequencing speed would be enormous. Allowing 30 nanoseconds to move a nozzle from one nucleotide to the next (a speed of about 1 cm/sec), it would take only 40 nanoseconds to sequence one base pair, which is equivalent to 1.5 Billion base pairs a minute.

[326] In certain embodiments, fast cooling techniques may be incorporated. As shown in Figure 27, for example, a specimen portion 2710 is within a probe system 2720 including a first probe 2730 and a cooling droplet supply nozzle 2740.

The cooling droplet supply nozzle 2740 may include liquid He, liquid N2, or other suitable coolant suitable for fast cooling application. The first probe may include any one of the above referenced types of probes. Alternatively, more than one probe may be used with a cooling droplet supply nozzle 2740, for example, for photonic application, current measurement, voltage bias, or other functionality as described herein.

[327] Referring now to Figure 28, another embodiment of the present invention is shown. A specimen portion 2810 is within a probe system 2820 including a first probe 2830 and a light nozzle 2850. The light nozzle 2850 and the first probe 2830 are activated, either sequentially, simultaneously or overlapping in time, to facilitate current detection, measurement, or other impact of the detection contribution effect, as described above. The first probe 2830 may include any one of the above referenced types of probes. The light nozzle 2850 may provide various types photonic energy, for example, visible, UV, X-Ray, THZ, IR, or FRIR.

[328] In other embodiments described herein, and referring back to Figures 6A-6F, the probes may be oriented at various angles with respect to the specimen. Referring to Figure 6A and 6B, all probes and probe sets described herein may be configured with respect to the specimen at various angles. For example, referring to Figure 6A, a probe set 630 may be oriented generally perpendicular (in the length direction) to a specimen 650. Further, referring to Figure 6B, a probe set 630 may be oriented (in the length direction) generally at an angle θ with respect to a specimen 650. Referring to Figure 6C, a system 660 is presented whereby the orientation of plural probe sets 630 relative a specimen 650 varies. Because the objects of the specimen 650 (e.g., bases within a DNA strand) may have different orientations, it may be desirable to

sequence with a plurality of probe sets 630. The plurality of probe sets 630 may have different angles θ_1, θ_2, θ_3, θ_4, θ_5,... θ_n (e.g., 20° to 160° in suitable increments, arranged sequentially, randomly or in another desirable arrangement. During measurement as described further herein, a controller may determine which orientation of the probe set yields the best signal for a particular base at its inherent orientation. This allows one to measure the data from the probe sets of the array, and determine the optimum signal for certain bases or groups of bases. In another embodiment, and referring to Figures 6D-6F, the angles of orientation in the height direction may also be varied. For example, referring to Figure 6D, probe set 630 may be oriented in the height direction generally perpendicular (90°) with respect to the specimen 650. Further, as shown in Figure 6E, probe set 630 may be oriented in the height direction generally at an angle ω with respect to the specimen 650. Referring to Figure 6F, a system 670 is presented whereby the orientation in a height direction of plural probe sets 630 relative a specimen 650 varies. Because the objects of the specimen 650 (e.g., bases within a DNA strand) may have different orientations, it may be desirable to sequence with a plurality of probe sets 630. The plurality of probe sets 630 may have different angles ω_1, ω_2, ω_3... ω_n (e. g., 20° to 160° in suitable increments, arranged sequentially, randomly or in another desirable arrangement. By measuring at these various angles, the opportunities for errors and misreading are minimized or eliminated.

[329] In another embodiment, and referring now to Figures {PFM}A and {PFM}B, a bendable membrane material {PFM}10 having a nano-scale probe attached thereto is provided. The nano-scale probe {PFM}12 may one of the aforementioned probes such as a known nucleotide strand, functionalized group, or other molecular

probe. Preferably the bendable membrane material {PFM}10 include a metallic surface with the probe {PFM}12 attached thereto to facilitate current measurement. Using a suitable MEMS device or other plunger {PFM}20, a flexible metal membrane {PFM}16 is pulsed to make contact with the specimen {PFM}40 to resolve it.

[330] As with the other probe types described herein, a 2D or 3D array may be provided. Further, these arrays may include homogeneous or heterogeneous probe types.

[331] Furthermore, in general, the probe may make contact with the assistance of other known devices such as angstrom or sub-angstrom precision actuators, MEMs devices, or other mechanical devices.

[332] Referring now to Figure {TS1}, a structure {TS1}05 is shown that facilitates attraction and transport polymeric structures such as DNA fragments, RNA molecules, proteins, or other polymeric structure. A substrate {TS1}10 is provided with one or more coaxing lines {TS1}20. These coaxing lines or regions may be in the form of channels, channels including a suitable coaxing material, lines or regions of the surface of the substrate {TS1}10 treated with a suitable coaxing material, a ridge or other protrusion defining the one or more coaxing lines {TS1}20, or a ridge or other protrusion defining the one or more coaxing lines {TS1}20 treated with a suitable coaxing material. A coaxing material may include materials such as amino-silane, biotin, other known bonding materials, charged conductive particles such as platinum, gold or other suitable material.

[333] In general, a the specimens may include magnetic portions, or suitable chromophores or fluorophores to help guide and manipulate the specimens.

[334] Note that the substrate {TS1}10 may be in the form of a glass slide, e.g., on the order of 1-2 cm by 3-5 cm. Alternatively, the substrate {TS1}10 may be in the form of a disc or wafer. The form factor of the slide will generally be a function of the analysis tools and/or manipulation tools used to work with the specimen.

[335] This structure {TS1}05 may be used with DNA sequencing tools, for example, described in conjunction with U.S. Patent Application Ser. No. 10/775,999 filed on February 10, 2004 entitled "Micro-Nozzle, Nano Nozzle and Manufacturing Methods Therefor", U.S. Provisional Patent Application Ser. No. 60/669,029 filed on April 7, 2005 entitled "DNA Sequencing Method and System", and U.S. Provisional Patent Application Ser. No. 60/699,619 filed on July 15, 2004 entitled "Molecular Analysis Probe, Systems and Methods, including DNA Sequencing", all of which are incorporated by reference herein.

[336] Further, these structures {TS1}05 may be used with various other types of analytical tools such as optical imaging tools. Certain useful optical imaging tools that may benefit from the structures {TS1}05 described herein are described in U.S. Patent Application Ser. No. 10/800,148 filed on March 12, 2004 entitled "Microchannel Plates And Biochip Arrays, And Methods Of Making Same" and U.S. Provisional Patent Application Ser. No. 60/674,012 filed on April 22, 2005 entitled "Microchannel Plate And Method Of Making Microchannel Plate", all of which are incorporated by reference herein.

[337] Referring now to Figure {TS2}, a structure {TS2}05 is shown that facilitates attraction and transport polymeric structures such as DNA fragments, RNA

molecules, proteins, or other polymeric structure. A substrate {TS2}10 is provided with a plurality of coaxing lines {TS2}20.

[338] Referring now to Figure {TS3}, a structure {TS3}05 is shown that facilitates attraction and transport polymeric structures such as DNA fragments, RNA molecules, proteins, or other polymeric structure. A substrate {TS3}10 is provided with one or more virtual coaxing lines {TS3}25 defined by plural electrodes {TS3}30 therealong. These virtual coaxing lines or regions may be in the form of channels with suitable electrodes {TS3}30, virtual lines or regions on the surface of the substrate {TS3}10 with suitable electrodes {TS3}30, a ridge or other protrusion defining the one or more virtual coaxing lines {TS3}20 with suitable electrodes {TS3}30. Accordingly, with plural discontinuous electrodes {TS3}03, the virtual coaxing line {TS3}25 is defined. The electrodes in these embodiments may include pre-charged particles, include an on-board battery, or include electrodes that are activated by suitable devices with the system reader.

[339] Referring now to Figure {TS4}, a structure {TS4}05 is shown that facilitates attraction and transport polymeric structures such as DNA fragments, RNA molecules, proteins, or other polymeric structure. A substrate {TS4}10 is provided with one or more coaxing lines {TS4}20 having plural electrodes {TS4}30 therealong. These coaxing lines or regions may be in the form of channels, channels including a suitable coaxing material, lines or regions of the surface of the substrate {TS4}10 treated with a suitable coaxing material, a ridge or other protrusion defining the one or more coaxing lines {TS4}20, or a ridge or other protrusion defining the one or more coaxing lines {TS4}20 treated with a suitable coaxing material, wherein the coating material may be

the same as those described above, or alternatively may include materials that have attraction forces when subjected to the electric fields created by the electrodes {TS4}30.

[340] In certain embodiments, an electric field may be applied at a desired start position {TS4}40 on the structure {TS4}05. Further, in the various embodiments of the structures that facilitate attraction and transport of specimens, various features may be aligned to other system features described herein.

[341] For example, and referring now to Figures {TS5}A-{TS5}G, a method of coaxing strands onto a structure {TS1}05, {TS2}05, {TS3}05 or {TS4}05. A structure {TS5}05 is inserted into a solution containing one or more polymeric structures such as DNA strands or fragments. One or more fragments will attach to said structure {TS5}05 as shown by arrows in Figure {TS5}C. Referring to Figures {TS5}D-F, structure {TS05}05 having one or more polymeric strands attached thereto is then pulled out of the liquid. Preferably, the structure {TS05}05 is removed in a direction along the axis of the coaxing line such that the liquid flow direction and gravity also contribute to the attractive forces of the coaxing lines. Accordingly, since the liquid flow forces, gravitational forces and the contribution of the coaxing line are in substantially the same direction, the strands are coaxed toward alignment. In certain embodiments, an electric field may be applied at a desired start position on the structure {TS05}05.

[342] To assist the denaturing in conjunction with the precise stepwise motion, the DNA strand can be straightened bay various methods. In one embodiment, electrostatic fields may be used to attract the negatively charged strands. In another embodiment, a magnetically attractive bead may be applied to an end of the DNA strand, and the strand pulled with magnetic force. In a further embodiment, viscosity

optimization may be employed, such that while dragging the strand through a liquid proximate or in the channel, it will straighten upon optimal dragging velocity and fluid viscosity conditions. Further, hydrophilicity may be used, e.g., by suitable material treatment at or in the nozzles and channel walls, to attract nucleotides. In other embodiment, hydrophobicity may be used, e.g., by suitable material at or in the nozzles and channel walls, to maintain the fluid within the channel.

[343] Referring now to Figure {CS1}, an overview of a coarse shuttle system {CS1}10 is shown. System {CS1}10 serves to facilitate displacement of the extended object {CS1}20, and in particular to move and stretch an extended object {CS1}20such as a DNA or RNA strand or fragment through a path {CS1}14 (which may be a channel or along the surface of a substrate) between two sides {CS1}30, {CS1}40.

[344] In general, each side {CS1}30, {CS1}40 has a plurality of electrode pairs arranged about the path {CS1}14. For example, as shown in Figure {CS1}, the channel 14 includes a wider opening area {CS1}16, for example, to increase the likelihood of extended object {CS1}20 encountering the channel {CS1}14. Electrode pairs {CS1}31, {CS1}41 through {CS1}38, {CS1}48 are arranged on the sides {CS1}30, {CS1}40. In the example where the extended object {CS1}20 is a negatively charged extended object, such as a DNA strand, positive charges are applied across the Electrode pairs {CS1}31, {CS1}41 through {CS1}38, {CS1}48, thereby coaxing the extended object {CS1}20 into and through the path {CS1}14.

[345] Note that the path {CS1}14 may be in the form of a channel, e.g., having partially enclosed walls such as a concave groove, V-shaped groove, U-shaped groove, or other suitable shape. Alternatively, the path {CS1}14 may instead be defined

by suitable surface treatment, as described further herein. Alternatively, .the path {CS1}14 may be an elevated ridge treated or pattered with electrodes, either along the sides as shown with respect to the molecular shuttle herein or along all or portions of the length of the path {CS1}14.

[346] Figures {NS1}A-C show an embodiment of a molecular shuttle {NS1}07, for example, for fine displacement of an extended object {NS1}12. In general, the molecular shuttle {NS1}07 may be used to controllably displace an extended object {NS1}12, for example, from a first location {NS1}16 to a second location {NS1}18 to a third location {NS1}20, and so on. The extended object {NS1}12, such as a DNA strand, DNA fragment, RNA molecule, protein molecule, or various other types of polymer and extended object, is typically charged, in this case shown as negatively charged. The molecular shuttle {NS1}07 includes a plurality of spatially opposing probes {NS1}22, {NS1}24 within or upon substrates or substrate regions 26, 28 thereby defining a path {NS1}30 therebetween. In certain preferred embodiments, these probes {NS1}22, {NS1}24 are formed as probes as described herein. As shown in Figure {NS1}A, the extended object {NS1}12 is outside of the path {NS1}30. By applying a positive charge at probes {NS1}22, {NS1}24 at the end of the molecular shuttle {NS1}07 (as indicated by "+" signs in Figure {NS1}A), the extended object {NS1}12 will be attracted to an opening {NS1}32 of the path {NS1}30.

[347] Referring to Figure {NS1}B, when another positive charge is applied through the probes {NS1}22, {NS1}24 at a location indicated by line {NS1}18, with negative charges provided by probes or electrodes between position {NS1}18 and the positive charge at opening {NS1}32, the extended object {NS1}12 will be attracted to the

position {NS1}18 within the channel path {NS1}30. Referring to Figure {NS1}C, the process is continued to shuttle the extended object {NS1}12, for example, to a position {NS1}20 within the path {NS1}30.

[348] Referring now to Figures {NS2}A-{NS2}D, a molecular shuttle {NS2}07 may be formed of various shapes, including but not limited to a curved or semicircle channel (Figure {NS2}A), a Y-shaped channel (Figure {NS2}B), a series of channels directed in a radial manner to or from a central point (Figure {NS2}C), or T-shaped (Figure {NS2}D), for example.

[349] Note that the path {NS1}30 may be in the form of a channel, e.g., having partially enclosed walls such as a concave groove, V-shaped groove, U-shaped groove, or other suitable shape. Alternatively, the path {NS1}30 may instead be defined by suitable surface treatment, as described further herein. Alternatively, .the path {NS1}30 may be an elevated ridge treated or pattered with electrodes, either along the sides as shown with respect to the molecular shuttle herein or along all or portions of the length of the path {NS1}30.

[350] Referring now to Figure 26, a reference position and precision nanometer metrology system is shown. A reference position probe (RPP), e.g., formed of platinum or other suitable material, or in the form of a nano-light guide, or other excitation probe structure, is included in the probe set or nanonozzle array set. The positions of each probe or nanonozzle relative the RPP is known. This reference position probe provides a known starting point when sequencing commences for precise metrology.

[351] Referring now to Figure 75, the stepped motion of ssDNA is shown relative to a known position of the RPP.

[352] In certain embodiments, the specimen may be within a channel of the base. A channel may include suitable fluid, or the specimen may be coaxed through a channel with little or no fluid.

[353] In other embodiments, the specimens may be embedded within the base, e.g., in a biochip.

[354] In certain embodiments, an electron or photon intensifier such as a micro-channel intensifier may be used. For example, referring to Figure 16A and 16B, these embodiments are shown.

[355] Referring to Figure 16A, the probe emitter interacts selectively with the specimen in an elastic or inelastic manner, whereby energy is lost, and the event lead to the release of photons or electrons that have specific energy indicative of the nature of the molecule or monomer. These electrons or photons may be too few to be measured directly. Therefore, the invention herein provides for an intensification or amplification sub-system such as micro-channel plate intensifiers known in the art, e.g., night vision goggles or photo-multipliers.

[356] Referring now to Figure 16B, where the probe is either metallic and/or a molecular probe, interaction with the specimen may be through inelastic tunneling current. Rather than measuring this tunneling current directly, it is possible to provide a sub-system for allowing either photons or electrons to be emitted. The photons or electrons to be emitted may occur upon a hybridization event, or by applying suitable voltage energy to emit inelastic electrons indicative of the spectra of the specimen. This

electron is also detected my an intensifier/amplification sub-system described above with respect to Fig. 16A.

[357] Referring now to Figure 16C, an array of intensifiers/amplifier sub-systems as described with respect to Figure 16A or 16B may be provided. For example, the exciting probe beams or other probe types may be tuned or optimized from a particular monomer, for example, in a DNA sequencing system, A, T, C, G, such that the electrons or photons are emitted are signatures of each type of nucleotide to be detected.

[358] Sequencing extended objects including but not limited to DNA, RNA, proteins in general, other polymers, oligomers, and other nano-scale structures. Thus, as shown and described, the herein system including nano-nozzles and nano-nozzle arrays are very well suited for ultra fast real time DNA sequencing operations.

[359] In addition to sequencing or analyzing DNA strands or fragments, probes and systems according to the present invention may be used for various types of extended objects including but not limited to DNA, RNA, proteins in general, other polymers, oligomers, and other nano-scale structures.

[360] Referring now to Figure {MAN1}, a probe {MAN1}02 having extremely small tip dimensions *t* (or array or set of such probes) may be used as a general purpose manipulator for manipulating materials on the molecular or atomic level. For example, using the probe {MAN1}02 provides for a high field strength, in part due to its symmetry. This high field that is advantageously localized due to the small probe dimensions will enable attraction of DNA strands, proteins, graphene layers, nanoparticles, other molecules, mono-molecular layers, or N such layers.

[361]

[362] Referring to Figure {LITH1}, a general system is depicted for using the herein probes for ultra high resolution nanolithography. A probe set may be provided, for example, wherein each probe includes the same or different materials. In further embodiments, three-dimensional nanostructures may be fabricated using the probes herein.

[363] Figures {NS1}A-C show an embodiment of a molecular shuttle {NS1}07. In general, the molecular shuttle {NS1}07 may be used to controllably displace an extended object {NS1}12, for example, from a first location {NS1}16 to a second location {NS1}18 to a third location {NS1}20, and so on. The extended object {NS1}12, such as a DNA strand, DNA fragment, RNA molecule, protein molecule, or various other types of polymer and extended object, is typically charged, in this case shown as negatively charged. The molecular shuttle {NS1}07 includes a plurality of spatially opposing probes {NS1}22, {NS1}24 within or upon substrates or substrate regions 26, 28 thereby defining a channel {NS1}30 therebetween. In certain preferred embodiments, these probes {NS1}22, {NS1}24 are formed as probes as described herein. As shown in Figure {NS1}A, the extended object {NS1}12 is outside of the channel 30. By applying a positive charge at probes {NS1}22, {NS1}24 at the end of the molecular shuttle {NS1}07 (as indicated by "+" signs in Figure {NS1}A), the extended object {NS1}12 will be attracted to an opening {NS1}32 of the channel.

[364] Referring to Figure {NS1}B, when another positive charge is applied through the probes {NS1}22, {NS1}24 at a location indicated by line {NS1}18, with negative charges provided by probes or electrodes between position {NS1}18 and the positive charge at opening {NS1}32, the extended object {NS1}12 will be attracted to the

position {NS1}18 within the channel. Referring to Figure {NS1}C, the process is continued to shuttle the extended object {NS1}12, for example, to a position {NS1}20 within the channel.

[365] Referring now to Figures {NS2}A-{NS2}D, a molecular shuttle {NS2}07 may be formed of various shapes, including but not limited to a curved or semicircle channel (Figure {NS2}A), a Y-shaped channel (Figure {NS2}B), a series of channels directed in a radial manner to or from a central point (Figure {NS2}C), or T-shaped (Figure {NS2}D), for example.

[366] Referring to Figure {AS1}, a method is shown to use the probes according to the present invention to create atomically smooth surfaces. For example, a probe {AS1}10 with an attached voltage source is swept over a surface {AS1}50. In the configuration of the probe as shown in Figure {AS1}, the probe produces a very high localized field strength. This field can be used to sweep a surface to make it atomically smooth.

[367] Another embodiment of the present invention exploits the ability to make atomically smooth ultra-thin films as taught in the present invention Figures {SLG 31B. These films can used as flexible substrates for analyzing or sequencing unknown specimens. As shown in Figure {AFTM1}, this flexible membrane may replace the flexible cantilevers in Figures {AFM1}-{AFM3}. Figure {AFTM1} shows a system {AFTM1}10 a membrane {AFTM1}12 between supports {AFTM1}14. As the specimen {AFTM1}30 passes under a probe {AFTM1}20, atomic interactions occur, generally as described above with respect to Figures {AFM1}-{AFM3}. However, the probes {AFTM1}20 are fixed, thus the membrane {AFTM1}12 is deflected by those atomic

forces. The deflection of the membrane {AFTM1}12, in response to atomic forces, is detected by measuring the reflection of the incident laser beam {AFTM1}40 on the membrane {AFTM1}12. By separating the deflection from the probe, a more general purpose apparatus results, namely, combing AFM capability with STM imaging as well sequencing tools all in one device. As shown in {AFTM1}, one or more probes {AFTM1}20 are connected to suitable voltage sources and the supports {AFTM}14. Other stimuli may also be provided for certain applications, such as scanning tunneling and other sequencing functionality. For specificity, the prove may be a specifically formed probe, such as a nucleotide specific probe as described above. A device particularly suited for sequencing DNA strands includes one that incorporates at least a set of 4 probes, include nucleotide specific probes for A,C,T and G, for example, in a configuration as described herein with respect to Figure {AFM2}, with the flexible membrane {AFTM1}12.

[368] This membrane deflecting apparatus allows for the possibility of replacing the laser beam with a parallel conducting plate directly underneath the membrane separated by an appropriate distance. As shown in Figures {AFTM2}A-{AFTM2}B, this forms a capacitance that varies according to the deflection of the membrane. Figures {AFTM2}A-{AFTM2}B show the deflection of the substrate membrane in response to the forces at different probe positions. Therefore, the capacitance value variation or modulation can be related to the atomic forces experienced by the membrane. This happens because the fixture holding the probes is held substantially fixed, thereby forcing only the membrane to respond to the forces.

[369] The capacitance value is designed to be in the range of 0.1 to 10 nano-Farad so that it can be part of a resonant circuit, Figure {AFTM2}C, comprising an inductance to oscillate at frequencies in the ranges of 10KHz- 1MHZ or 1MHz to several GHz. By coupling an tunable sweep oscillator, it is possible to monitor the power absorbed by the system as a function of frequency.

[370] Fig.{AFTM2}D shows the intensity, I_ω may be plotted as a function of frequency , $\omega = (LC)^{1/2}$, for different probe positions. Measuring frequency shifts can be related to the capacitance variation that results from the varying forces F_ω at different positions. Figure {AFTM2}E illustrates the dependence of the F_ω on the frequency for attractive and repulsive forces. In a first position, the probe experiences a repulsive force, causing the capacitance to decrease, and shifting the frequency to ω_1. In a second and third probe positions, the forces are attractive, shifting the requires upward to ω_2 and ω_3 respectively.

[371] Figure {AFTM3}A illustrates yet another embodiment of the present invention whereby a tool for analyzing specimens including specific application of sequencing DNA , RNA and atomic force imaging is provided. A probe according the teachings of the present invention is attached to a flexible membrane or cantilever. According to the exploded view in Fig. {AFTM3}B, a first thin film inductor connected to a first thin film plat of a capacitor are deposited on the flexible membrane on the surface opposite the probe. A second thin film inductor connected to a second thin film plate of a capacitor are deposited on a rigid member on the surface facing the flexible membrane. The rigid member and flexible membrane and attached to each other with a suitable spacer having a thickened that determines a desired capacitance value. The

spacer also may contain an integrated circuit for processing and/or analyzing the signals which result from the interaction of the probe with the specimen. This signal is manifested in the variation of the capacitance as a result of the forces that cause the membrane deflection. Figure {AFTM3}C shown the circuit model for analysis and processing. Similar detection principles apply as those employed in the apparatus described Figures {AFTM2}A to {AFTM2}E.

[372] This integrated atomic force probe can be used as in conventional ATM modes, as well as for sequencing. The latter is accomplished by sequentially inserting different integrated probes functionalized to specify different nucleotides. Alternatively, it is preferred to integrate several capacitive probes in a single structure to perform parallel sequencing and analysis functions as shown in Figure {AFTM4}. This fully integrated system allows the flexibility to have probes of different shapes and functionalized to recognize predetermined certain specimens. The system can be addressed to select one of many modes, including but not limited to STM, AFM, sequencing, magnetic analysis, or other suitable functionalities, because it has a unique activation/deactivation feature. This is accomplished with an integrated circuit that supplies a DC voltage to the plates of the capacitor that is selected to deactivate. This causes the flexible membrane to be attached permanently to the upper rigid plate. The removal of the DC voltage releases the membrane and selects it and its probe for activation.

[373] Figure {AFTM5} illustrates the system of Figure {AFTM4} further including nucleotide specific probes for increases specificity, for example, particularly suitable for imaging, analyzing and sequencing DNA specimens.

[374] The fully integrated probe illustrated in Figures {AFTM3}-{AFTM5} can be advantageously manufactured by the methods and systems described in Applicant's multi-layered manufacturing methods, as described in U.S. Non-provisional Application Serial Nos. 09/950,909, filed September 12, 2001 entitled "Thin films and Production Methods Thereof"; 10/222,439, filed August 15, 2002 entitled "MEMs And Method Of Manufacturing MEMs"; 10/017,186 filed December 7, 2001 entitled "Device And Method For Handling Fragile Objects, And Manufacturing Method Thereof"; PCT Application Serial No. PCT/US03/37304 filed November 20, 2003 and entitled "Three Dimensional Device Assembly and Production Methods Thereof"; U.S. Patent No. 6,857,671 granted on April 5, 2005 entitled "Method of Fabricating Vertical Integrated Circuits"; U.S. Non-provisional Application Serial Nos. 10/717,220 filed on November 19, 2003 entitled "Method of Fabricating Muti Layer MEMs and Microfluidic Devices"; 10/719,666 filed on November 20, 2003 entitled "Method and System for Increasing Yield of Vertically Integrated Devices"; 10/719,663 filed on November 20, 2003 entitled "Method of Fabricating Muti Layer Devices on Buried Oxide Layer Substrates"; all of which are incorporated by reference herein. However, other types of semiconductor and/or thin film processing may be employed.

[375] While the above examples apply to the sequencing of DNA, it is appreciated that the probes can be functionalized to have the ability to recognize other molecules with precise specificity making these methods more general for the recognition and analysis on unknown chemicals. It will have applications not only as a scientific tools, but also for medical as well as for sensing hazardous materials.

[376] It is known that the replication and transcription of DNA involves the separation of the two strands to reveal the base sequence of the single stand to be replicated or transcribed. This is accomplished with a helicase enzyme which causes the complementary strands to separate in a first position to complete the transcription or replication processes. When this is completed, the two complementary strands bind again and the helicase separates them at a second adjacent position to repeat the process. This is repeated along the entire DNA length until the replication or transcription is done.

[377] The present invention which teaching the analysis of an extended object in general, and a single DNA strand sequence in particular, may be extended to also sequence double strand specimens. This may be accomplished according the embodiments of the present invention by causing the nano-probe to interact with the nucleotide bases in the major and minor groves of the helical structure of the DNA strand or fragment. This process may optionally be facilitated further by the use of a suitable catalyst or enzyme such as helicase to cause local separation of the complementary strands to reveal the bases to be sequenced and to cause them to interact optimally with the nano-probe as described herein. The catalyst or enzyme may be attached to or dispensed from the analyzing nano-probe or attached to or dispensed from an auxiliary nano-probe or nano-funnel in close proximity to the analyzing nano-probe. Except for this additional step using the catalyst, the procedure to analyze the double stranded DNA is carried out using the embodiments taught herein for analyzing the single strand DNA.

[378] While preferred embodiments have been shown and described, various modifications and substitutions may be made thereto without departing from the

spirit and scope of the invention. Accordingly, it is to be understood that the present invention has been described by way of illustrations and not limitation.